EVALUATION OF GASTRIC FUNCTION IN PRIMATES FOLLOWING THE ADMINISTRATION OF AGENTS WHICH ACT AT THE KAPPA RECEPTOR

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Title of Thesis: Evaluation of Gastric Function in Primates Following the Administration of Agents Which Act at the Kappa Receptor

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Title of Dissertation: The Effect of Kappa Receptor Agonists on Gastric Emptying and Secretion in Primates

Patricia Lynn Touzeau: Doctor of Philosophy, 1988.

Dissertation directed by: Terez Shea-Donohue, Ph.D., Assistant Professor of Medicine and Physiology, USUHS.

ABSTRACT

The physiological effects of kappa agonists on gastric function have not been fully elucidated. In the present studies, the effect of kappa receptor agonists (dynorphin(1-13), U50,488H and ketocyclazocine), a putative antagonist of the kappa receptor (MR1452 MS), and a non-specific opiate antagonist (Naloxone), on gastric emptying and secretion were evaluated alone and in combination. A marker dilution technique was used to measure, concurrently, gastric fractional emptying rate and secretion in conscious, chair-adapted rhesus monkeys during a fasting period and following the intragastric administration of an 80 ml water load (pH 7.4, 37° C). All values listed as significant had a p value of less than 0.05.

The opioid agonists utilized in these studies inhibited gastric emptying. Unlike agonists that are active

at mu (morphine) and delta (enkephalins) receptors, selective kappa agonists (dynorphin-(1-13); U50,488H) did not alter acid secretion, while the kappa/mu agonist, ketocyclazocine, significantly decreased it. Dynorphin significantly decreased Na^+ output from 14 to 8 uEq/min during fasting and following the water load from 22 to 9 uEq/min. The highest dose of ketocyclazocine significantly suppressed postload Na^+ from 21 to 9; K^+ output from 4 to 1.2 and Cl^- from 36 to 16 uEq/min and fluid from 0.34 to 0.14 ml/min. U50,488H had no effect on these parameters, indicating a possible functional specificity among opioid receptor subtypes. Low doses (0.05, 0.1 ug/kg/min) of the antagonist, MR1452 MS, did not affect gastric emptying or secretion, but doses greater than 0.25 ug/kg/min did significantly inhibit emptying from 9.3 to 2.2 %/min during the first ten minutes postload and significantly increased postload Cl^- output from 24 to 44 uEq/min and fluid output from 0.21 to 0.33 ml/min. Although MR1452 MS was unable to block any of the agonists during the fasting period, it significantly antagonized the inhibitory effect of dynorphin-(1-13) during the later phase of gastric emptying, restoring the rate from 1%/min to the control level of 3.5%/min. MR1452 MS also significantly antagonized the early phase of ketocyclazocine's inhibition of emptying, returning the postload fractional emptying rate from 1.3%/min to 4.1%/min. Naloxone was an ineffective antagonist of the selective kappa

receptor agonist U50,488H, but was able to antagonize significantly ketocyclazocine's inhibition of gastric emptying and restored the emptying rate from 1.3%/min to 5.7%/ min. These results suggest that kappa receptor stimulation inhibits gastric emptying and non-parietal cell secretion and that selective kappa agonists have no action on parietal cells.

THE EFFECT OF KAPPA RECEPTOR AGONISTS ON GASTRIC EMPTYING AND SECRETION IN PRIMATES

by

Patricia Lynn Touzeau

Dissertation Submitted to the Faculty of the
Department of Physiology
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requirements for the degree of
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DEDICATION

To my father, A.T. Touzeau, M.D., through whom I first became interested in science; he has given me his loyal support and encouragement during the course of this work.

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BACKGROUND

Opiate Receptors and Endogenous Opiates

The term opiate is derived from the Greek word for juice, opium. Opium is a drug produced from the dried resin of the opium poppy, Papaver somniferum, and it has been recognized since ancient times not only as an antidiarrheal drug, but also as an analgesic and addictive agent. In 1806, a German named Sertürner isolated and described an opiate alkaloid which he called morphine, after Morpheus, the Greek god of dreams. This discovery prompted others to search for natural and synthetic compounds which would produce the therapeutic effects without the addictive potential of morphine.

Some of the earliest scientific studies describing the mechanism of the effect of opiates in dogs were carried out by Plant and Miller (1926). They were the first to demonstrate that morphine caused constipation by stimulating contraction of the circular muscle in the intestine. Schaumann (1957) and Paton (1957) hypothesized that the antitransit effect of morphine and related compounds was related to a suppression of acetylcholine release from longitudinal muscle preparations and suggested that opiates acted directly on neurons via an unknown mechanism. Subsequently, all opioid agonists were found to share similar stereochemical properties and were antagonized by chemical-

ly-related compounds; thus, it was postulated that opiates act at specific sites (receptors) (Becket and Casey, 1954). Binding to opiate receptors in the brain was first demonstrated by Pert and Snyder (1973). Using rats, mice and guinea pigs, they showed stereospecific, naloxone-reversible binding to receptors on brain membranes. Since they could not demonstrate binding in intestine stripped of myenteric plexus, they concluded that opiate receptors were present only in nervous tissue. However, this idea was proven later to be incorrect. During this time, several other laboratories independently reported the presence of binding sites for morphine (Wuster et al., 1971; Terenius, 1972; 1973; Simon et al., 1973; Wong and Hirning, 1973) and the concept of the receptor became a reality.

The idea of multiple types of opiate receptors was introduced by Martin and his co-workers (1976). Using morphine-dependent dogs, he observed that the opioid antagonist, nalorphine, antagonized the effect of morphine and also acted as an analgesic. Based on this information, he proposed that an opiate's effects were mediated by more than one receptor. In subsequent studies, he also demonstrated that different analogues of morphine produced three distinct patterns of behavior in dogs. Thus, he proposed that the actions of these drugs were due to their activity at three, separate receptors and called them mu, kappa and sigma (Gilbert and Martin, 1976; Martin et al., 1976).

The fact that morphine, a plant alkaloid, bound to receptors in the brain and had physiological effects, encouraged the search for endogenous ligands of opiate receptors. The first identification of endogenous opioids was made by Hughes and co-workers (1975), who isolated two pentapeptides from porcine brain tissue that had morphine-like activity in the isolated guinea-pig ileum bioassay. They called these peptides met- and leu-enkephalin from the Greek word enkephalos, meaning "in the head." Subsequently, numerous other endogenous opioid peptides not only were isolated and characterized (Li et al., 1965; Hughes et al., 1975; Goldstein et al., 1975; Cox et al., 1975; Li and Chung, 1976) but also were shown to have discrete distributions throughout the brain, central nervous system and peripheral tissues (Linnoila et al., 1978; Akil et al., 1984; Vincent et al., 1984; Cox, 1985).

Subsequent to the discovery of enkephalins, two other distinct families of endogenous opioid peptides have been identified: endorphins and dynorphins. These families are derived from large, precursor molecules, and radioimmunoassay and molecular cloning techniques have demonstrated that each precursor, proenkephalin, pro-opiomelanocortin and prodynorphin, is the product of a single gene. The precursors are the source of a number of peptide sequences, which serve as peptide hormones, neurotransmitters or paracrines (Douglass et al., 1984; Cox, 1985). While the

peptides are cleaved from distinct precursors, they have three properties in common: 1) each peptide tends to have selective affinity for a particular receptor subtype; 2) all of the known opioid peptides contain the same recognition signal, the N-terminal sequence for the enkephalins (Tyr-Gly-Gly-Phe-Met or Leu); and 3) they are antagonized by the non-specific opiate antagonist, naloxone. Antagonism by naloxone is considered to be the criterion which proves that a putative opiate has activity at opiate receptors (Goldstein and Cox, 1978).

The precursor molecules contain the sequences for a number of different peptides; which one is actually produced depends upon the tissue or organ in which the precursor is situated. The peptide precursor molecule, prodynorphin, contains five peptides which are carboxy-terminal extensions of leu-enkephalin: Dynorphin 32, which is cleaved enzymatically into Dynorphin A (dynorphin-(1-17) and Dynorphin B (rimorphin), alpha and beta neo-endorphin as well as several smaller peptides. While Dynorphin A (D17), Dynorphin B and alpha-neo-endorphin act predominantly at the kappa receptor, like the other peptides derived from prodynorphin they have been shown to have some activity at mu and delta receptors (Cox, 1988). Prodynorphin and its related peptides have been demonstrated throughout the brain and central nervous system and in many peripheral tissues by immunocytochemical

and histological techniques (Watson et al., 1981; Feurle et al., 1982; Spampinato and Goldstein, 1983; Hedner and Cassuto, 1987). The prodynorphin gene product having the highest affinity for kappa receptors, dynorphin-(1-17) is distributed in discrete brain regions, the anterior and posterior lobes of the pituitary gland as well as in all regions of the gastrointestinal tract (Weber et al., 1982; Cox, 1988).

It should be noted that outside of the brain and central nervous system, the highest concentration of immunoreactive dynorphin is found in the stomach, intestine and upper jejunum (Spampinato and Goldstein, 1983). Dynorphin-like immunoreactivity was first demonstrated in the gastrointestinal tract by Tachibana and associates (1981), who isolated it from porcine duodenum. Subsequently, dynorphin-like immunoreactivity was isolated in fibers in the myenteric and submucous ganglia, circular muscle layer and in endocrine cells throughout the gastrointestinal tract (Bitar and Mahklouf, 1985; Sundler et al., 1987). In addition, alpha-neo-endorphin and dynorphin-(1-17)-like immunoreactivity has been demonstrated in the gastrointestinal tract in human tissues (Maysinger et al., 1982).

Exogenous Opiates

The finding that opiate receptors and their endogenous ligands are present in the gastrointestinal tract made

them likely candidates for use as antidiarrheal agents in humans, and much research has been carried out with this goal in sight. Since endogenous opiates are difficult to isolate in quantity and tend to be metabolized rapidly (Ho et al., 1980; Leslie and Goldstein, 1982; Hughes,1983), synthetic analogues were developed that were highly selective for a particular receptor subtype. Two synthetic analogues which have activity at kappa receptors are the benzomorphans, ketocyclazocine and ethylketocyclazocine, which are structurally similar to natural morphine. Each of these agents, like morphine, has an aromatic ring structure containing five asymmetric carbon atoms, with an -OH in the 3' position, is the (-) stereoisomer, and has a nitrogen atom ionized at physiological pH. Ketocyclazocine and ethylketocyclazocine (Porreca et al., 1983; 1984) were used in many early studies to evaluate kappa receptor function. They were later found to have activity at both kappa and mu receptors and to share some of the characteristic properties of morphine (Gillan and Kosterlitz, 1982; Maurer, 1982; Ward and Takemore, 1983).

U50,488H is the prototype of a series of structurally novel opioids synthesized by Upjohn (Lahti et al., 1982; VonVoightlander et al., 1983) as an outgrowth of research to develop compounds which possessed the analgesic properties of morphine without the addictive side effects. Its structural characteristcs are also similar to those of morphine.

As it did not cause morphine-like physical dependence, it was hoped that this synthetic opiate compound could be used clinically as an antidiarrheal agent. They determined that U50,488H was antagonized *in vitro* by the general opiate antagonist, naloxone, as well as by the putative kappa receptor antagonist, MR2266. In addition, it was found subsequently to have a higher affinity for the kappa receptor than either dynorphin-(1-13) or ethylketocyclazocine *in vitro* and *in vivo* (Lahti et al., 1982; VonVoightlander et al., 1983), and it has been a useful experimental tool for studying effects mediated by kappa opiate receptors.

Regulation of Gastrointestinal Motility by Opiates

The widespread distribution of opioids and their receptors throughout the central nervous system and peripheral tissues has made the identification of the site(s) controlling opioid-mediated gastrointestinal transit difficult. Opiates have been reported to act at supraspinal (Margolin, 1963; Stewart et al., 1977; Schulz et al., 1979; Galligan and Burks, 1982), spinal (Porreca et al., 1982; Porreca and Burks, 1983; Vaught et al., 1983; Koslo et al., 1985); and peripheral sites (Manara and Bianchetti, 1985; Tavani et al., 1979; Fiocchi et al., 1982; Notarnicola et al., 1983; Perrachia et al., 1984). There is evidence that the inhibitory action of morphine on gastrointestinal motility may occur at all three sites. Margolin (1963) first

observed that morphine injected into the lateral ventricles of rats inhibited gastrointestinal transit following spinal cord transection, adrenalectomy or vagotomy. Since the observed inhibition of motility was not blocked by the peripherally-acting antagonist, n-methyl naloxone, he concluded the inhibition was mediated centrally. Later studies demonstrated that mu agonists also exerted their effect following intrathecal injection (Porreca et al., 1983). In opposition to early studies suggesting a predominantly central action of mu agonists, their ability to act at peripheral sites was demonstrated by Stewart and associates (1977), who reported that subcutaneously administered morphine suppressed intestinal transit in both vagotomized and non-vagotomized rats. A peripheral effect was also supported by the study of Bianchi and associates (1982) which showed that naltrexone, an opioid antagonist that does not cross the blood-brain barrier, completely reversed the inhibition of gastrointestinal transit induced by intravenous morphine. Finally, these findings were expanded and corroborated by Perrachia et al. (1984) who found that intravenously administered morphine was blocked by n-methyl naloxone.

Inhibition of gastrointestinal transit is produced by compounds other than mu receptor agonists such as morphine. Enkephalin analogues, specific for delta and/or mu receptors, have also been shown to inhibit gastrointestinal

transit when administered centrally (Bueno et al., 1985). In contrast, the endogenous ligands have no effect when given centrally. This lack of an effect has been attributed to the longer half-life of the synthetic compounds, as endogenous peptides are more likely to be degraded enzymatically before they can produce an effect. Thus, despite the activity of the enkephalin analogues, endogenous enkephalins probably do not alter gastrointestinal motility via a central mechanism. It has been shown, however, that several delta receptor agonists (Porreca and Burks, 1983) modulate gastrointestinal transit at spinal sites.

The effects on gastrointestinal transit of peripherally administered opioid agonists active at delta receptors are contradictory. It has been shown that the delta receptor agonists, met-enkephalin and [D-Ala^2]Met-enkephalinamide (DMET), a synthetic analogue, inhibit gastric emptying in primates (Shea-Donohue et al., 1983) and that FK-33-824, another enkephalin analogue, inhibited gastrointestinal transit when injected intravenously into rats (Feretti et al., 1981). In contrast, Shook and co-workers (1987) recently demonstrated that the highly selective delta receptor agonist, [D-Pen^2,D-Pen^5]enkephalin (DPDPE), had no effect when injected subcutaneously in rats. These discrepancies may be due to properties of the analogues such as half-life, the dose and/or the species utilized.

In early studies using rats and mice, Porreca's

group (1983) demonstrated that the putative kappa receptor agonists, ketocyclazocine and ethylketocyclazocine, dose-dependently inhibited gastrointestinal transit after peripheral administration. The peripheral action of ethylketocyclazocine on gastrointestinal transit was further investigated by Ward and Takemore (1982), who used a highly selective, reversible mu receptor antagonist, beta-funaltrexamine, to occupy mu receptors in mice and then observed the effects of peripherally or centrally injected ethylketocyclazocine. In these studies, they found that ethylketocyclazocine dose-dependently inhibited gastrointestinal transit when injected subcutaneously, but had no effect after central administration. They concluded from these results that kappa agonists regulate gastrointestinal transit via a peripheral mechanism. However, since both of these agonists have activity at mu receptors (Gillan and Kosterlitz, 1982; Ward and Takemore, 1982), they further (1983) postulated that the inhibition of gastrointestinal transit produced by these drugs is probably due to activity at peripheral mu, and not kappa, receptors. Their hypothesis is supported by recent work investigating the separation of peripheral and central antitransit effects of mu agonists which has been carried out by Shook and co-workers (1987) who demonstrated that morphine acts at peripheral opiate receptors in sub-analgesic (<1 mg/kg s.c.) doses, and at both central and peripheral receptors in the analgesic doses (>1 mg/kg s.c.)

in rats. Thus, possibly like morphine, low doses of ketocyclazocine activate only peripheral mu receptors.

Later studies confirmed that kappa receptor agonists such as ketocyclazocine or U50,488H had no effect on gastrointestinal motility after central administration in mice or rats (Porreca et al., 1983; 1984). Furthermore, the kappa receptor agonists ketocyclazocine, dynorphin-(1-9) and dynorphin-(1-13) did not affect gastrointestinal transit when injected intrathecally, suggesting that this action on gastrointestinal motility is not mediated at the level of the spinal cord (Porreca and Burks, 1983). Finally, peripherally administered selective kappa receptor agonists have been reported to have no effect on gastrointestinal motility. Hirning et al. (1985) showed that kappa receptor agonists did not stimulate intestinal contractions in the dog ex vivo, and U50,488H was also shown to have no effect on gastrointestinal transit in rats (Porreca et al., 1984; Shook et al., 1987).

Gastric Acid Secretion

The reported effects of opiates on acid secretion have been contradictory. Morphine, the classic mu receptor agonist, has long been known to have opposite effects on acid output since it has been shown both to inhibit or stimulate acid secretion in a variety of species (Hedner and Cassuto, 1987; Cox, 1988). In addition, the delta agonists,

met- and leu-enkephalin and their analogues were found to both inhibit and stimulate acid secretion (Konturek et al., 1978; 1980; Feldman et al., 1980; Feldman and Cowley, 1982; Ferri et al., 1984; Shea-Donohue et al., 1983; Kostritsky-Pereira et al., 1984; Sullivan, 1984). Finally, the kappa receptor agonist dynorphin-(1-17) decreased total acid output (Ferri et al., 1984), while U50,488H, a selective, synthetic kappa ligand, was reported recently to increase acid output (Fox and Burks, 1988).

Opiates may influence gastric acid secretion by altering the release of acetylcholine which, in turn, could modify the H^+ output of parietal cells or the release of gastrin from antral G cells. It was demonstrated early on by both Paton (1957) and Schaumann (1957) that morphine inhibited the release of acetylcholine from cholinergic neurons supplying guinea pig ileum muscle strips. Thus, opiates could alter gastric acid secretion by modulating cholinergic activity directly by inhibiting cholinergic neurons or indirectly via inhibitory interneurons. This latter suggestion is supported by the work of Konishi et al. (1984), who demonstrated that enkephalins act presynaptically in parasympathetic ganglia to suppress acetylcholine release, resulting in a decrease in the amount of gastrin released. Since gastrin stimulates acid secretion from parietal cells, an inhibition of gastrin would decrease gastric acid secretion.

One mechanism by which opiates may affect acetylcholine-mediated acid secretion is by modifying the ionic conductances of action potentials (Cherubini and North, 1985). Mu agonists are known to increase K^+ conductance in myenteric neurons, while kappa agonists ultimately decrease Ca^{++} influxes. Both of these actions decrease presynaptic acetylcholine release from the myenteric neurons, which could affect gastric function in several ways. Inhibition of acetylcholine release could alter gastric emptying by inhibiting the vagally-mediated cholinergic response to distention and secretion. In addition, acetylcholine stimulates acid secretion directly by acting on the parietal cell and indirectly by causing the release of gastrin. Any reduction in acetylcholine available to these cells results in decreased acid secretion. Another way in which opiates could modulate gastric secretion is by regulating Na^+/K^+ ATPase activity. Gastric secretion of fluid and electrolytes is mediated by electrogenic pumps whose catalyst is Na^+/K^+ ATPase. If these membrane transport systems are interrupted, transport cannot occur. It has been demonstrated recently that the kappa receptor agonists dynorphin-(1-17) and ethylketocyclazocine inhibit Na^+/K^+ ATPase activity in cardiac cells, and opiates could, therefore, also mediate Na^+/K^+ ATPase in gastric cells. This effect on cardiac muscle cells was not antagonized by naloxone. Thus, the observed inhibition may be due to a non-opiate effect of

these drugs or to differences in ATPase found in diverse species and tissues. Alternatively, the lack of naloxone antagonism could be the result of the well-documented insensitivity of kappa receptor agonists to naloxone (Maeda et al., 1988). It remains to be determined, therefore, if opiates mediate Na^+/K^+ ATPase in gastric cells.

It is not clear whether the effects of opiates on acid secretion are mediated centrally or peripherally. A recent study by Fox and Burks (1988) suggested that gastric acid secretion in rats is decreased by both central and peripheral mu receptors. In addition, they found acid secretion was unaltered by delta receptors, and increased by kappa receptor selective agonists. Based on a series of pharmacological studies in rats, they concluded that kappa agonists produce their effects by a mechanism stimulating the release of acetylcholine from enteric neurons that, in turn, stimulates cholinergic M1 receptors located on the parietal cell to increase acid secretion.

Gastric Secretion of Ions and Fluid

There is little data regarding the effects of opiates on gastric ion and fluid secretion. Shea-Donohue et al. (1983) showed that met-enkephalin and one of its synthetic analogues, [D-Ala^2]Met-enkephalinamide, inhibited Na^+, Cl^-, and fluid secretion in primates. There is a good deal of experimental data, however, showing that opioids are

important modulators of fluid and electrolyte transport in the small intestine and colon.

Morphine stimulates Na^+ and water absorption in dogs, probably as a result of increased mucosal blood flow in response to morphine (Mailman, 1980; 1984; Rees et al., 1986). In addition, morphine and loperamide, another mu agonist, which is active peripherally, have been demonstrated to inhibit PGE_1-stimulated fluid secretion. However, only morphine blocked the PGE_1-mediated increase in cAMP. As the loperamide suppression of fluid secretion was also not antagonized by naloxone (Beubler and Lembeck, 1980), it may exert its effects beyond the cAMP cascade or may not be mediated via an opiate receptor.

Finally, both morphine and enkephalins are known to stimulate duodenal alkalai secretion (Flemstrom et al., 1985; Rees et al., 1986). This effect was naloxone reversible, and it was proposed that it was due to an activation of the $HCO3^-/Cl^-$ exchange mechanism (McKay et al., 1981). Thus, a large body of data suggests that endogenous opiates may play a role in the modulation of gastric secretion.

Summary

The data reviewed above suggest that exogenous opiates affect the gastrointestinal tract by acting on receptors located either on neurons, smooth muscle or secretory cells. In addition, endogenous opioids, when used as

drugs, have actions that are similar to those of exogenous opiates. The widespread distribution of endogenous opioids and their receptors have been found in high concentrations throughout the gastrointestinal tract make it likely that endogenous opioids play physiological roles in the normal function of the gastrointestinal tract. Nevertheless, knowledge of the effects of stimulation of opioid receptors by their endogenous ligands in the gastrointestinal tract has yet to be fully elucidated. Most of the work investigating the actions of opiates on gastrointestinal function has been carried out using delta and mu receptor agonists. Consequently, less was known about the effects of kappa agonists and their receptors on gastric function, especially secretion. Therefore, these studies were undertaken to evaluate the role of kappa receptor agonists on gastric emptying and secretion in primates.

RATIONALE

It has been demonstrated that mu, delta and kappa receptors and their endogenous ligands are present throughout the gastrointestinal tract, and their physiological importance in gastrointestinal function is an area of continuing study. There is evidence that central and peripheral activation of mu and delta receptors inhibits gastric emptying and has contradictory effects on acid secretion, but the contribution of kappa receptors is unclear. Thus, the studies presented here were designed to explore the hypothesis that kappa receptor agonists modulate gastric emptying and secretion.

Previous work using the putative, synthetic kappa receptor agonist, ketocyclazocine, demonstrated inhibition of gastrointestinal motility in mice and rats, while the synthetic, selective kappa receptor agonist U50,488H, had no effect. These conflicting data concerning kappa receptors led to the design of the research presented here.

Experiments were conducted using conscious, chair-adapted monkeys because the most meaningful answers to physiological questions can be produced by studies carried out on intact, conscious animals. The unique value of primates as experimental subjects is their close, phylogenetic relationship to humans.

These studies explored the effects of kappa receptor agonists dynorphin-(1-13), U50,488H and ketocyclazocine alone and in combination with the opioid antagonists MR1452 MS and naloxone on gastric emptying and secretion. All the agonists and antagonists utilized were given by a continuous, subcutaneous infusion which is somewhat analogous to hormonal secretion. We administered an intragastric water load, as a mild, vagally-mediated distention stimulus, to produce changes in motility and secretion.

The first group of experiments were designed to determine the effects of kappa agonists on reflexly-induced gastric activity. In order to determine if the effects of kappa agonists were actually mediated via the kappa receptor, the agonists U50,488H or ketocyclazocine were infused in combination with a putative kappa receptor antagonist, MR1452 MS or the general opiate antagonist, naloxone. Secondly, if the effect of a kappa agonist could be blocked by a general antagonist, such as naloxone, the effect was indeed mediated by an opiate receptor. In another series of experiments, only a kappa receptor antagonist was infused. If gastric function were significantly affected by a kappa antagonist alone, then this would indicate the involvement of endogenous kappa receptor ligands in the control of the stomach.

MATERIALS AND METHODS

All studies were performed on conscious, chair-adapted rhesus monkeys (Macaca mulatta), weighing 3-8 kg. To habituate the animal to the study procedure, each monkey was intubated twice a week for one month with a #12 French double lumen nasogastric tube (Ventrol Levin; Mallinckrodt, Argyle, NY: bore 4mm; wall thickness, 1 mm). Experiments were performed twice a week. The day before the experiment, the food was removed from the monkeys' cages between 1 and 3 p.m. and they were fasted overnight. All studies took place between 9 a.m. and 12 p.m. the following day. On the morning of the study, an animal was removed from his cage, placed in a primate restraining chair, and taken to the laboratory. There, the nasogastric tube was inserted gently into one nostril and advanced through the esophagus until a slight resistance was felt indicating that the tube was situated in the most dependent part of the stomach. The tube was then marked on the portion closest to the nostril to be certain that the tube would remain in the same position throughout the entire study. The stomach contents were aspirated and the proper positioning of the tube was verified by injecting 15 ml of water and recovering the total volume (Dubois et al., 1977).

The tube was then secured at the nostril and at the top of the head with surgical tape. A catheter adapter

attached to a 3-way stopcock was connected to the end of the tube. The monkey's calf was then swabbed with alcohol and a 25 g butterfly needle (Mediwing infusion set, St. Louis, MO) was inserted subcutaneously (s.c.) into one or both legs and held in place by surgical tape. The butterfly infusion set was attached to a length of extension tubing. The tubing was connected to a syringe containing the drug which was placed in an infusion pump (Harvard Apparatus, South Natwick, MA). The chair holding the monkey was placed in a closed, ventilated, lighted booth and the nasogastric tube was drawn through a hole in the top of the booth and secured with tape. The initial subcutaneous infusion was then begun and continued for an equilibration period. All animals were studied during a 40-min fasting period and for 60 min after the administration of an 80 ml water load (pH 7.4; 37°C). Thus, the total time of the study was 100 minutes. The drugs, treatment of the samples, and technique used to evaluate gastric parameters are described below. The activities, doses and sources are listed in Table 1. Dose responses were determined for all kappa agonists and antagonists, as well as in studies where agonist and antagonist interaction was evaluated, to select the appropriate dose to be utilized in each series of experiments. The agonists used were an endogenous ligand of the kappa receptor, dynorphin-(1-13) (D13), a selective kappa receptor ligand, U50,488H (U50), and a putative kappa receptor agonist,

Table 1. Summary of the agents studied. Listed are the descriptions of the reported activity, dose and source.

Table 1

Drug	Action	Dose	Source
Dynorphin-(1-13)	Kappa agonist	2.2, 4.4 ug/kg/min	Sigma Chemical St. Louis, MO
U50,488H	Kappa agonist	0.05, 0.5, 1.0 ug/kg/min	The Upjohn Co. Kalamazoo, MI
Ketocyclazocine	Mu/kappa agonist	0.1, 0.15, 0.2, 1.0 ug/kg/min	Sterling Winthrop Rensselaer, NY
MR 1452 MS	Kappa antagonist	0.05, 0.1, 0.25 ug/kg/min	Boehringer-Engelheim Ridgefield, CT
Naloxone hydrochloride	Opioid antagonist	40 ug bolus followed by 4 ug/kg/min	Sigma Chemical St. Louis, MO

ketocyclazocine (KETO). The antagonists used were MR1452 MS (MR), a putative, highly selective kappa antagonist and naloxone (NAL), a recognized general antagonist of opioid receptors (Sawynok et al., 1979; Young and Woods, 1982). The drugs were reported to be 98-99% pure by HPLC (personal communication from suppliers).

The drugs were prepared as follows. On the day of the experiment, dynorphin-(1-13) was dissolved in a solution of 0.5% methanol and 0.5% 1M HCl (1:1) and diluted in saline (25 ml) prior to infusion. Ketocyclazocine was dissolved in 85% lactic acid to make a stock solution of 30 mg/ml and stored at 4°C. U50,488H was diluted in saline to a concentration of 100 ug/ml, aliquotted and stored at -40°C. On the day of the experiment, the aliquot was warmed gently to 37°C and sonicated. MR1452 MS was diluted in saline to a concentration of 0.03 ug/100 ml and frozen at -20°C in 1 ml aliquots. Naloxone was stored in a brown bottle in the laboratory. Naloxone and the other drugs stored as stock solutions were diluted to the appropriate concentration on the day of the experiment. Plasma gastrin (G17) levels were determined after the equilibration period in the studies using ketocyclazocine (0.2 ug/kg/min). In some studies using the agonists, heart rate was determined before the equilibration period and at the end of the study.

Each study consisted of 3 periods: 1) equilibration, 2) fasting, and 3) after the intragastric administration of

a water load. A control (0.9% saline diluent) or drug infusion (0.11 ml/min) was begun before the initiation of each study to establish steady concentrations of the drug in each animal. On separate days, each animal received a continuous, subcutaneous infusion of the drug alone or in combination. In the studies using naloxone, an intravenous bolus was administered and the subcutaneous infusion started immediately thereafter.

Determination of Gastric Function and Analysis of Samples of Gastric Juice

A dye-dilution technique (phenolsulfonphthalein, PSP), previously described and validated in humans and monkeys (Dubois et al., 1977; Shea-Donohue et al., 1983), was used to determine concurrently gastric emptying and ion and fluid output. Gastric emptying is presented during the fasting period as the mean gastric fractional emptying rate (%/min); gastric fractional emptying in response to the water load is presented as the fraction of the load remaining in the stomach over time (%) and is also divided into early and late phases of emptying in response to the water load. Output of ions was expressed as uEq/min.

Twenty minutes after the placement of the naso-gastric tube, a 60 ml syringe was attached and the contents of the stomach were mixed by repeated aspiration and reinjection for one minute. The amount of dye injected and,

therefore, the size of the aspirated sample was proportional to the volume of the gastric juice. For example, during a normal fasting period, the intragastric volume is approximately 5 - 10 mls. Thus, after the first mixing, 2.5 ml were aspirated and placed in a glass test tube which was covered with parafilm. Any remaining gastric juice was returned to the stomach. Then 5 ml of PSP were added to the stomach and the mixing process was repeated. Again 2.5 ml of the mixed gastric contents were then aspirated, retained, and the remaining gastric juice was returned to the stomach. Thus, since the total aspirated volume was equal to the injected volume of PSP, there was no net alteration in gastric volume. This process was repeated every 10 minutes during the fasting period. During the water-stimulated period, this procedure was repeated using larger volumes of PSP solution (10-20 mls) at 5 and 10 min after the infusion of the meal and every 10 min for the following 50 min. The samples of PSP and samples of gastric juice were centrifuged. Samples of the clear supernatant were adjusted to pH 10 with 0.25% Na_3PO_4 and analyzed for the concentration of PSP using a spectrophotometer at 560 nm (Gilford Macrosample, Oberlin, OH). Intragastric volumes of fluid (V_1, V_2,...) and mass of PSP (P_1, P_2,...) were determined using the dye-dilution principle (Hildes and Dunlop, 1951; Dubois et al., 1977). The assumptions and mathematical description of the dye-dilution technique which permits the simultaneous

determination of gastric emptying, fluid and ion outputs, has been described in the paper by Dubois and associates (1977). The method requires a thorough mixing of dye with gastric fluid, and assumes that a loss of dye mass reflects gastric emptying, while a reduced concentration of dye indicates gastric secretion. The mathematical assumptions for these determinations are outlined briefly below.

Fractional emptying rate (FER) is defined as the mls of the gastric contents emptied (ml/min) divided by the volume in the stomach (ml) times 100. Fractional emptying rate (g) is determined for each 10 min interval (t) between two dilutions, assuming that emptying is a first order (exponential) process during short intervals and uses the following equation.

$$g = -\log_e (P_2/P_1)/t \qquad (1)$$

Net rate of fluid output was then determined for the corresponding interval assuming that it remained constant over the given interval and uses the equation:

$$R_v = [V_2 - V_1 \cdot \exp(g.t)] \cdot g/[1 - \exp(g.t)] \qquad (2)$$

Intragastric fluid volumes and masses of PSP were then recalculated, taking into account the first estimate of

fractional emptying and fluid output, which were in turn recalculated (Dubois et al., 1977).

Hydrogen ion (H^+) output was measured in the clear supernatant of gastric samples by end-point titration to pH 7.4 with 0.02 N NaOH (Titration Assembly, Radiometer, Copenhagen, Denmark). Sodium (Na^+) and potassium (K^+) concentrations were measured using a flame photometer (Instrumentation Laboratories Model 443, Lexington, MA), and chloride (Cl^-) concentration was determined using an amperometric technique (Corning 920 M chloride meter, Medfield, MA). The intragastric mass of each ion (I_1, I_2,...) were determined. Net rate of ion output was then calculated by multiplying intragastric volumes by intragastric ion concentration. The equation used is:

$$R_1 = [I_2 - I_1 \cdot \exp(g.t) \cdot g/[1 - \exp(-g.t)] \quad (3)$$

The calculations were performed using a locally developed program and a PDP-10 computer (Division of Computer Research Technology, National Institutes of Health, Bethesda, MD). The assumptions involved have been described elsewhere (Dubois et al., 1977), and are based on the original contribution of Hildes and Dunlop (1951). However, in contrast to their method, the present technique allows correction for emptying and secretion occurring during the 1

min dye dilution interval and can be applied during fasting as well as following a water load.

Statistics

The means of each of the periods were averaged for each monkey, and these were, in turn, averaged together and the means and standard errors were determined for each group. The significance of differences observed for each measurement of gastric function (e.g., fractional emptying rate, fluid output, etc.) was evaluated using a three factor (treatment, time, and monkey) analysis of variance with repeated measures on the last two factors (time and monkey), followed by a t-test designed to evaluate differences among multiple means. The program LDU-040, and an IBM 370 computer (Division of Computer Research Technology, National Institutes of Health, Bethesda, MD) were used to perform the calculations.

EXPERIMENTAL RESULTS

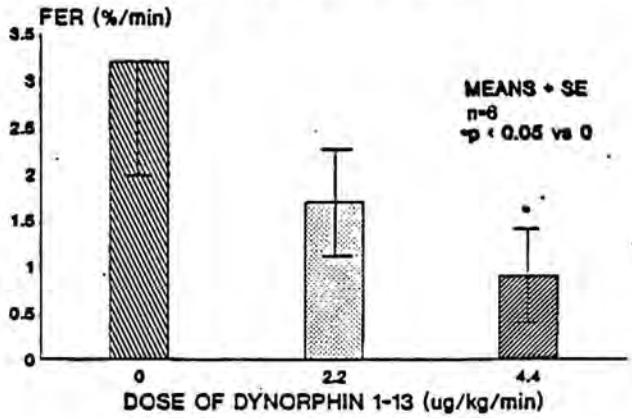
The Effect of Exogenously Administered Kappa Receptor Agonists on Gastric Emptying

All the kappa receptor agonists studied inhibited gastric emptying. The effect of an endogenous ligand of the kappa receptor, dynorphin-(1-13) (D13), at 2.2 and 4.4 ug/kg/min on mean fasting gastric fractional emptying rate (FER) is demonstrated in Figure 1 (Panel A). FER decreased significantly by 64% during the 4.4 ug/kg/min dose. Following the placement of water in the stomach, the inhibitory effect of D13 is also observed when emptying is expressed as the percentage of the load remaining in the stomach over time (Figure 1: Panel B). Each point represents the mean of the number of monkeys in the study (N=5,6 or 7). Compared to control, the higher dose of D13 (4.4 ug/kg/min) increased the percentage of the load remaining in the stomach throughout the entire period. To further evaluate the alterations in gastric emptying over time, the water load-stimulated FER was divided into two periods. In control animals, the early phase, 0 - 10 minutes, is the time of the greatest emptying response of the stomach to the administration of a water load. Figure 1 (Panel C) illustrates the mean FER for each of the defined periods. The low dose of D13 (2.2 ug/kg/min) did not alter FER during either period, but 4.4 ug/kg/min D13 did significantly inhibit FER during both phases. More-

Figure 1. The effect of Dynorphin-(1-13) (D13) on gastric emptying. Panel A illustrates mean fractional emptying rate (FER) during the fasting period; Panel B, the fraction of the water load remaining in the stomach over time and Panel C, mean postload FER from 0 to 10 minutes and 10 to 60 minutes.

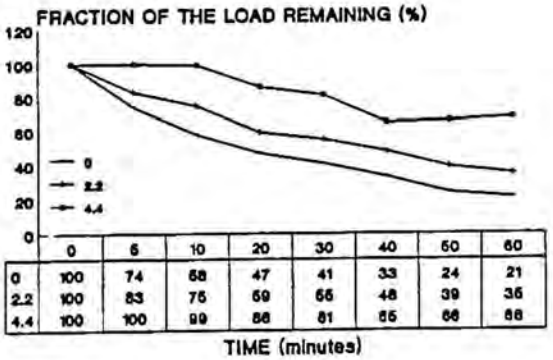
PANEL A

EFFECT OF DYNORPHIN 1-13 ON
FASTING FRACTIONAL EMPTYING RATE.



PANEL B

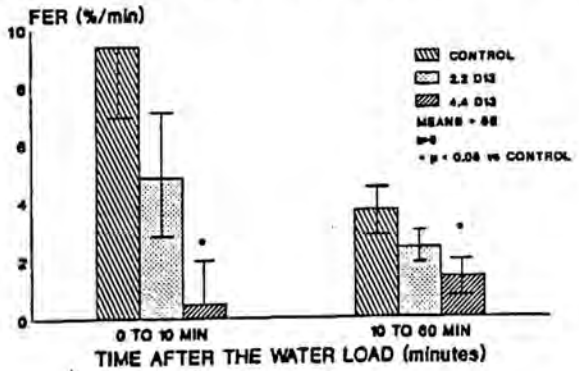
EFFECT OF DYNORPHIN 1-13
ON GASTRIC EMPTYING



	0	5	10	20	30	40	50	60
0	100	74	68	47	41	33	24	21
2.2	100	83	75	59	55	48	39	36
4.4	100	100	99	86	81	65	66	66

PANEL C

EFFECT OF DYNORPHIN 1-13 ON
FRACTIONAL EMPTYING RATE OVER TIME



over, the suppressive action of D13 on FER was greater in the first 10 minutes (97%) than in the subsequent 50 minutes (62%).

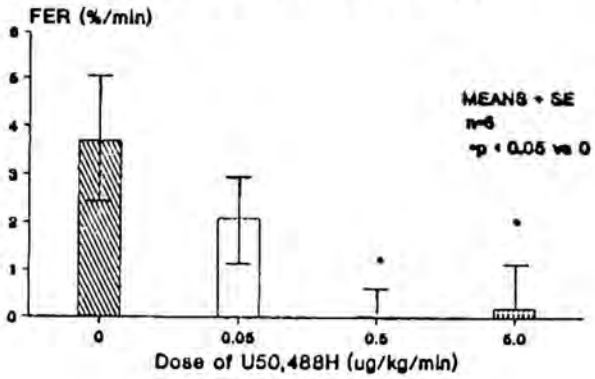
Figure 2 illustrates the effects of U50,488H on gastric emptying. Mean FER was significantly inhibited by 0.5 and 5.0 ug/kg/min of U50 during the fasting period (Panel A). During the postload period, these doses of U50 also appear to increase the percentage of the load remaining in the stomach (Panel B). These data are quantitated in Figure 2 (Panel C), showing the FER for U50 during the first 10 minutes and the last 50 minutes after the water load. The two highest doses of U50 significantly decreased the FER during both periods, while 0.05 ug/kg/min of dose of U50 had no effect during either period. Like D13, the suppressive action of 0.5 ug/kg/min of U50 was greater during the first 10 minutes (94%) than in the later phase (43%). The highest dose of U50, 5.0 ug/kg/min, produced a similar response during both the early and later phase (61% and 78% respectively).

The effect of ketocyclazocine (KETO) on mean gastric fasting FER is shown in Figure 3 (Panel A). When compared to control, all doses of KETO produced a significant reduction in fasting FER ranging from 56 to 92%. The inhibitory action of KETO on FER after the administration of the water load is illustrated in Figure 3 (Panel B) showing the percentage of the load remaining in the stomach over time. All

Figure 2. Effect of U50,488H (U50) on gastric emptying. Panel A illustrates the mean fractional emptying rate (FER) during the fasting period; Panel B, the fraction of the water load remaining in the stomach over time, and Panel C, the mean postload FER from 0 to 10 minutes and 10 to 60 minutes.

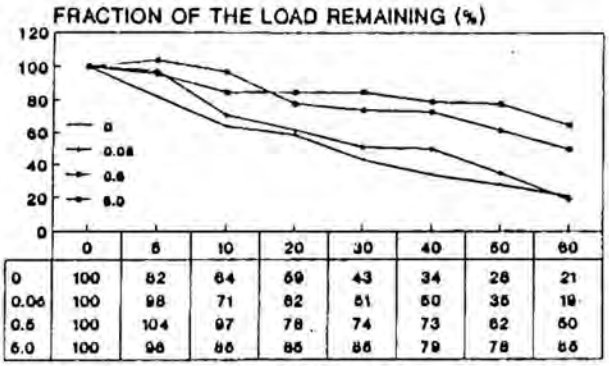
PANEL A

EFFECT OF U50,488H ON FASTING FRACTIONAL EMPTYING RATE



PANEL B

EFFECT OF U50,488H ON GASTRIC EMPTYING



	0	5	10	20	30	40	50	60
0	100	92	84	69	43	34	28	21
0.05	100	98	71	82	61	50	35	19
0.5	100	104	97	78	74	73	62	50
5.0	100	96	86	86	86	79	78	66

PANEL C

EFFECT OF U50,488H ON FRACTIONAL EMPTYING RATE OVER TIME

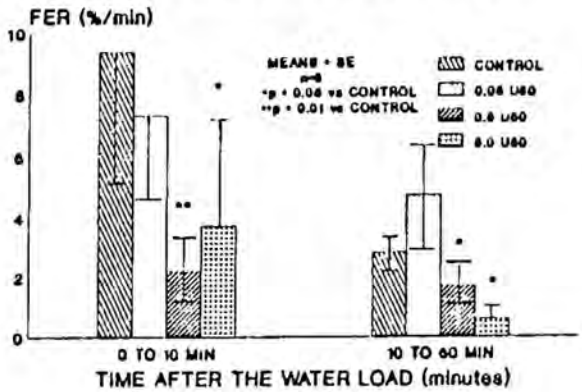
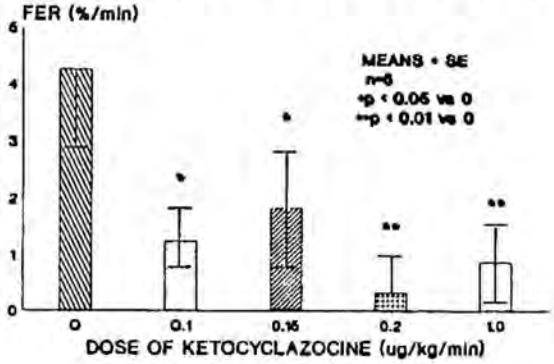


Figure 3. Effect of ketocyclazocine (KETO) on gastric emptying. Panel A illustrates mean fractional emptying rate (FER) during the fasting period; Panel B, the fraction of the water load remaining in the stomach over time, and Panel C, mean postload FER from 0 to 10 minutes and from 10 to 60 minutes.

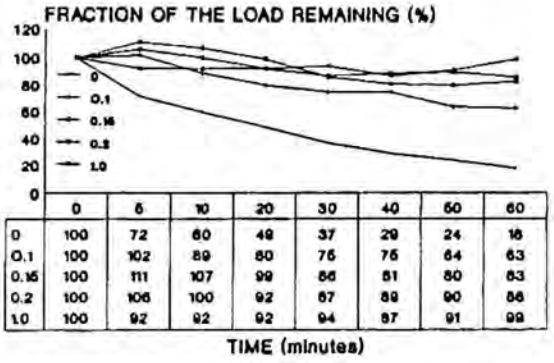
PANEL A

EFFECT OF KETOCYCLAZOCINE ON FASTING FRACTIONAL EMPTYING RATE



PANEL B

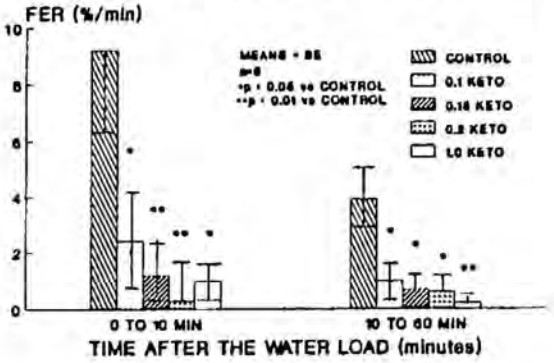
EFFECT OF KETOCYCLAZOCINE ON GASTRIC EMPTYING



	0	5	10	20	30	40	50	60
0	100	72	60	49	37	29	24	18
0.1	100	102	89	80	76	76	64	63
0.15	100	111	107	99	86	81	80	83
0.2	100	106	100	92	87	89	90	86
1.0	100	92	92	92	94	87	91	99

PANEL C

EFFECT OF KETOCYCLAZOCINE ON FRACTIONAL EMPTYING RATE OVER TIME



doses of KETO appeared to delay the gastric emptying during the entire period. This effect is confirmed in Figure 3 (Panel C), showing the FER during the first 10 minutes versus the last 50 minutes. All doses of KETO significantly suppressed FER during both the early and later phases of the gastric emptying response to a water load.

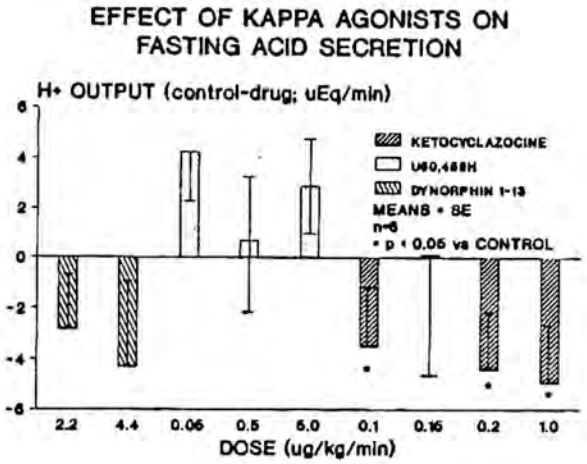
The Effect of Kappa Receptor Agonists on Acid Secretion

The effect of the three kappa agonists, D13, U50 and KETO, on gastric H^+ secretion during the fasting and post-load periods is presented in Figure 4. When compared to control, neither 2.2 nor 4.4 ug/kg/min of D13 had any significant effect on H^+ output during the fasting period or after the water load. As shown in Figure 4, U50 did not significantly alter H^+ during either period. The 0.05 ug/kg/min dose enhanced H^+ output in 2 of the 6 animals studied, resulting in a somewhat greater mean H^+ output during the water load-stimulated period.

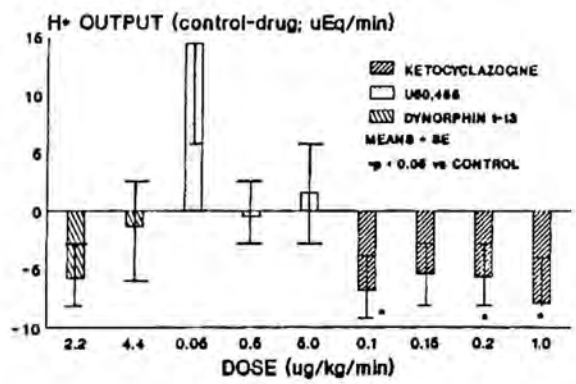
In contrast, 0.1, 0.2, and 1.0 ug/kg/min of ketocyclazocine significantly decreased H^+ output during both periods (52%-56%; $p<0.05$). Compared to control, plasma gastrin levels in response to 0.2 ug/kg/min of KETO (72 ± 5 pg/ml vs 71 ± 9 pg/ml) remained unchanged. An infusion of 0.15 ug/kg/min of KETO produced an average 50% reduction in acid secretion, ($p<0.06$). Since the doses of ketocyclazocine immediately above (0.2 ug/kg/min) and below (0.1

Figure 4. Effect of kappa agonists on acid secretion. Data are expressed as the mean change from control (control-drug). The direction of the change is then reversed for clarity so that a decrease from control is in the negative direction while an increase from control is in the positive direction. Panel A illustrates the mean change from control during the fasting period; and Panel B the mean change from control during the postload period.

PANEL A



PANEL B



ug/kg/min) the 0.15 ug/kg/min dose gave a significant reduction in acid, this result is likely due to experimental variation.

The Effect of Kappa Receptor Agonists on Fluid and Ion Outputs

Values for the ion and fluid outputs obtained during the fasting period and following the intragastric administration of water periods in response to 2.2 or 4.4 ug/kg/min of D13 are presented in Table 2. The Na^+ output was decreased significantly by the high dose of D13 (4.4 ug/kg/min) during both periods. Fluid, K^+ and Cl^- outputs were not modified significantly by D13 during either period.

Table 3 demonstrates the effect of 0.05 and 0.5 ug/kg/min of U50 on fluid and ion outputs. Neither dose of U50 significantly altered fluid, Na^+, or K^+ output during these studies. Fasting Cl^- output was significantly increased by the 0.05 ug/kg/min of U50.

Table 4 presents gastric fluid, Na^+, K^+ and Cl^- outputs in response to KETO. Fasting fluid output was decreased significantly by the highest dose of KETO, while fluid output was suppressed significantly by all doses of KETO following the intragastric instillation of water. The Na^+ output was not altered by KETO during the fasting period but was suppressed significantly by 0.2 and 1.0 ug/kg/min after the water load. In addition, all doses of KETO

Table 2. The effect of Dynorphin-(1-13) (D-13: 2.0 and 4.4 ug/kg/min) on gastric Na^+, K^+, Cl^- and fluid outputs. Values are means $\pm$ SE; n = 7. *$p<0.05$ when compared to control infusions using a three-factor analysis (treatment, time, and monkey) of variance with repeated measures on the last two factors.

Table 2

	s.c. Infusion (ug/kg/min)	Na^+ output (uEq/min)	K^+ output (uEq/min)	Cl^- output (uEq/min)	Fluid output (ml/min)
Fasting	0	13.8 ± 2.5	2.9 ± 0.4	24.5 ± 3.5	0.14 ± 0.03
	2.2 D13	9.6 ± 0.7	3.7 ± 0.9	19.5 ± 4.7	0.16 ± 0.04
	4.4 D13	8.1 ± 1.9*	2.0 ± 0.4	16.0 ± 2.1	0.09 ± 0.02
Postload	0	21.5 ± 7.5	4.0 ± 0.7	36.3 ± 4.5	0.34 ± 0.07
	2.2 D13	11.4 ± 1.2	3.7 ± 0.3	22.0 ± 3.3	0.32 ± 0.06
	4.4 D13	8.7 ± 0.7*	2.6 ± 0.4	23.6 ± 2.1	0.15 ± 0.05

Table 3. The effect of U50,488H (U50: 0.05 and 0.5 ug/kg/min) on gastric Na^+, K^+, Cl^- and fluid outputs. Values are means $\pm$ SE; n = 6. *p<0.05 when compared to control infusions using a three-factor analysis (treatment, time, and monkey) of variance with repeated measures on the last two factors.

Table 3

	s.c. Infusion (ug/kg/min)	Na^+ output (uEq/min)	K^+ output (uEq/min)	Cl^- output (uEq/min)	Fluid output (ml/min)
Fasting	0	17.3 ± 3.8	6.7 ± 0.7	14.9 ± 1.1	0.15 ± 0.02
	0.05 U50	22.1 ± 3.7	5.3 ± 0.8	31.9 ± 4.2*	0.21 ± 0.03
	0.50 U50	14.3 ± 2.9	4.3 ± 0.6	22.4 ± 1.6	0.14 ± 0.02
Postload	0	16.9 ± 2.3	4.0 ± 0.2	26.1 ± 2.2	0.25 ± 0.04
	0.05 U50	21.5 ± 2.0	8.0 ± 1.0	36.9 ± 5.1	0.43 ± 0.09
	0.50 U50	26.9 ± 2.1	5.6 ± 0.3	36.6 ± 2.9	0.30 ± 0.06

Table 4. The effect of ketocyclazocine (KETO: 0.1, 0.15, 0.2 and 1.0 ug/kg/min) on gastric Na^+, K^+, Cl^- and fluid outputs. Values are means $\pm$ SE; n = 7. *p <0.05 or ** p<0.01 when compared to control infusions using a three-factor analysis (treatment, time, and monkey) of variance with repeated measures on the last two factors.

Table 4

	s.c. Infusion (ug/kg/min)	Na^+ output (uEq/min)	K^+ output (uEq/min)	Cl^- output (uEq/min)	Fluid output (ml/min)
Fasting	0	13.8 ± 2.5	2.9 ± 0.4	24.5 ± 3.5	0.14 ± 0.03
	0.10 KETO	12.8 ± 3.5	2.9 ± 0.7	13.5 ± 5.7	0.13 ± 0.03
	0.15 KETO	14.2 ± 1.2	3.0 ± 0.4	22.3 ± 0.8	0.18 ± 0.04
	0.20 KETO	10.3 ± 2.2	2.4 ± 0.4	12.1 ± 3.6	0.11 ± 0.02
	1.00 KETO	8.5 ± 1.3	1.5 ± 0.3	15.0 ± 5.8	0.08 ± 0.02**
Postload	0	21.5 ± 7.5	4.0 ± 0.7	36.3 ± 4.5	0.34 ± 0.07
	0.10 KETO	14.5 ± 2.9	2.3 ± 0.5*	13.2 ± 2.0**	0.10 ± 0.04*
	0.15 KETO	10.1 ± 0.8	1.6 ± 0.1**	25.9 ± 7.9	0.10 ± 0.08*
	0.20 KETO	9.2 ± 1.4*	2.1 ± 0.3**	14.0 ± 2.4**	0.11 ± 0.08*
	1.00 KETO	8.7 ± 1.4*	1.2 ± 0.1**	16.0 ± 3.4**	0.14 ± 0.07*

significantly inhibited K^+ output during this time. During the postload period, Cl^- output decreased and was significant for all but the 0.15 ug/kg/min dose.

The Effect of a Putative Kappa Receptor Antagonist, MR1452 MS, on Gastric Emptying and Secretion

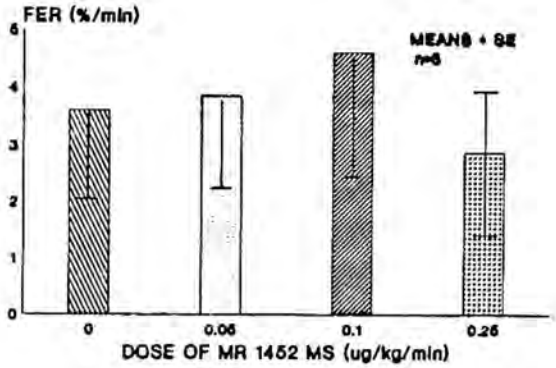
Figure 5 illustrates the effect of a putative selective antagonist of the kappa receptor, MR1452 MS (MR), on gastric emptying. When compared to control, MR had no significant effect on fasting mean FER (Panel A). The effect of MR on gastric emptying of the water load is demonstrated in Figure 5 (Panel B) showing the percentage of the load remaining in the stomach over time. This figure shows that gastric emptying following 0.05 and 0.1 ug/kg/min was nearly identical to that of control. In addition, 0.25 ug/kg/min appears to slow emptying during the first 10 minutes in a manner similar to that of the kappa agonists (Figures 1-3). Panel C in Figure 5 shows the FER during the first 10 minutes and for the last 50 minutes. As suggested in Figure 5 (Panel B), 0.25 ug/kg/min of MR significantly inhibited FER during the early phase but had no effect during the later period.

The effects of MR on electrolyte and fluid outputs are presented in Table 5. The values for fasting and water load-stimulated H^+ output were not significantly different from those of control. In contrast, fluid output was en-

Figure 5. The effect of MR1452 MS (MR) on gastric emptying. Panel A illustrates the mean fractional emptying rate (FER) during the fasting period; Panel B, the fraction of the load remaining in the stomach over time, and Panel C, mean post-load FER from 0 to 10 minutes and 10 to 60 minues.

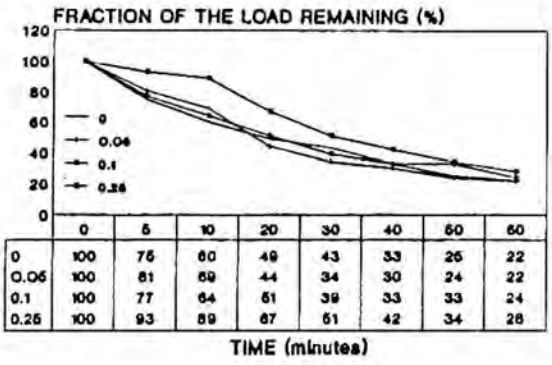
PANEL A

EFFECT OF MR 1452 MS ON FASTING FRACTIONAL EMPTYING RATE



PANEL B

EFFECT OF MR1452 MS ON GASTRIC EMPTYING



	0	5	10	20	30	40	50	60
0	100	75	60	49	43	33	25	22
0.05	100	81	69	44	34	30	24	22
0.1	100	77	64	51	39	33	33	24
0.25	100	93	89	67	51	42	34	28

TIME (minutes)

PANEL C

EFFECT OF MR 1452 MS ON FRACTIONAL EMPTYING RATE OVER TIME

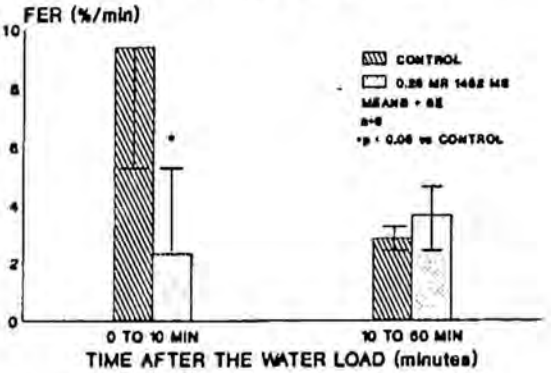


Table 5. The effect of MR1452 MS (MR: 0.05, 0.1, and 0.25 ug/kg/min) on gastric H^+, Na^+, Cl^- and fluid outputs. *$p<0.05$ when compared to control infusions using a three-factor analysis (treatment, time, and monkey) of variance with repeated measures on the last two factors.

Table 5

	s.c. Infusion (ug/kg/min)	H^+ output (uEq/min)	Na^+ output (uEq/min)	K^+ output (uEq/min)	Cl^- output (uEq/min)	Fluid output (ml/min)
Fasting	0	7.8 ± 1.8	17.3 ± 3.8	6.7 ± 0.7	14.9 ± 1.1	0.15 ± 0.02
	0.05 MR	14.0 ± 8.6	18.2 ± 5.6	5.0 ± 1.2	31.8 ± 10.6	0.30 ± 0.07*
	0.10 MR	14.1 ± 8.0	16.2 ± 2.7	3.9 ± 0.7	31.6 ± 6.8	0.20 ± 0.04
	0.25 MR	13.3 ± 8.1	17.0 ± 1.2	5.3 ± 0.5	36.6 ± 9.2	0.30 ± 0.07*
Postload	0	12.4 ± 5.3	16.9 ± 2.3	4.0 ± 0.2	23.5 ± 5.3	0.21 ± 0.03
	0.05 MR	13.6 ± 6.2	21.4 ± 3.4	5.2 ± 0.6	31.6 ± 5.2	0.32 ± 0.30*
	0.10 MR	10.5 ± 5.1	16.2 ± 1.4	3.9 ± 0.3	23.9 ± 2.1	0.23 ± 0.03
	0.25 MR	19.3 ± 6.5	19.8 ± 3.6	5.6 ± 0.6	43.7 ± 2.9*	0.33 ± 0.06

hanced significantly by 0.05 and 0.25 ug/kg/min during both periods. No dose of MR had a significant effect on Na^+ or K^+ output during the fasting period or following the water load. Fasting Cl^- output was not significantly altered by MR. However, Cl^- output was significantly enhanced by the highest dose of MR after the intragastric administration of water.

The Effect of Kappa Receptor Agonists in Combination with MR 1452 MS on Gastric Emptying

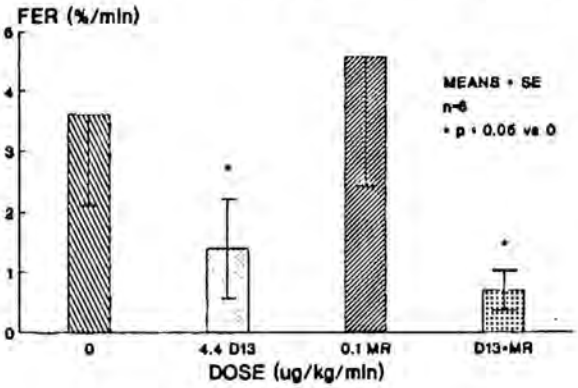
In order to demonstrate that the effects produced by the drugs utilized in these studies were mediated by the kappa receptor, we used the putative kappa antagonist, MR, in an attempt to block the changes in gastric function induced by the agonists. Figure 6 shows the effect of 0.1 ug/kg/min of MR on the inhibition of gastric emptying following 4.4 ug/kg/min of D13. The MR had no effect on the suppression of mean fasting FER induced by D13 (Panel A). The effect of MR on FER following the water load in response to D13 is illustrated in Figure 6 (Panel B), showing the percentage of the load remaining in the stomach over time. While MR was unable to overcome the inhibiitory effect of D13 during the first 10 minutes, it completely antagonized the action of D13 from 10 to 60 minutes.

MR did not antagonize the suppressive action on FER produced by U50 during either period. This lack of an

Figure 6. The effect of 4.4 ug/kg/min Dynorphin-(1-13) (D13) and 0.1 ug/kg/min MR1452 MS (MR), alone and in combination, on gastric emptying. Panel A illustrates the mean fractional emptying rate (FER) during the fasting period; Panel B, the fraction of the load remaining in the stomach over time, and Panel C, the mean postload FER from 0 to 10 minutes and 10 to 60 minutes.

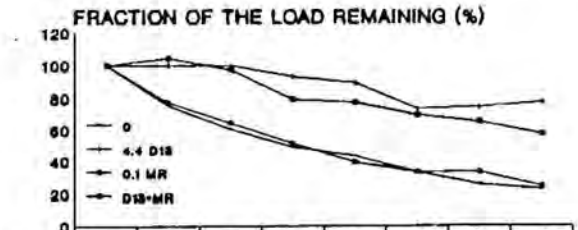
PANEL A

EFFECT OF DYNORPHIN 1-13 AND MR 1452
ON FASTING FRACTIONAL EMPTYING RATE



PANEL B

EFFECT OF DYNORPHIN 1-13 AND MR1452
ON GASTRIC EMPTYING

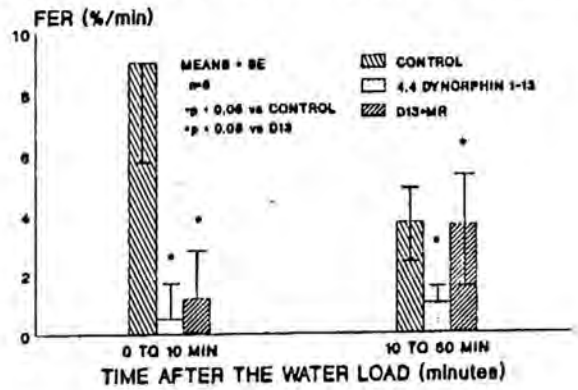


	0	5	10	20	30	40	50	60
0	100	75	60	49	43	33	25	22
4.4 D13	100	100	100	93	89	73	74	77
0.1 MR	100	77	64	51	39	33	33	24
D13+MR	100	104	97	79	77	69	66	57

TIME (minutes)

PANEL C

EFFECT OF DYNORPHIN 1-13 AND MR 1452 MS
ON FRACTIONAL EMPTYING RATE OVER TIME



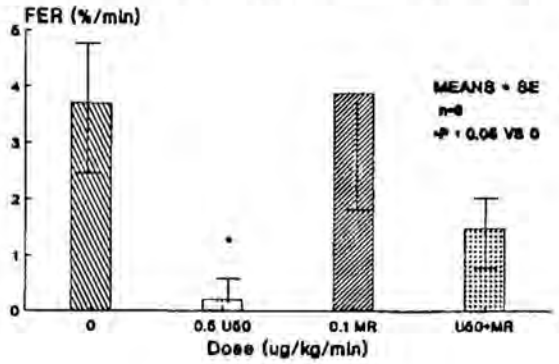
effect is demonstrated in Figure 7 (Panels A and B) which shows the mean fasting FER and the percentage of the load remaining in the stomach over time. The values for the gastric emptying of the water load are nearly identical for U50 alone and U50 + MR. This is confirmed in Figure 7 (Panel C) depicting FER over time. The FER after the load was significantly suppressed by both U50 or U50 + MR during that entire period, showing that MR was unable to antagonize U50.

In the final series of studies in this section, KETO (0.15 ug/kg/min) was administered with 0.1 ug/kg/min of MR. (Figure 8). MR was not able to block the inhibitory effect of KETO on fasting FER (Panel A). However, following the water load, the inhibitory effect of KETO appeared to be attentuated by 0.1 ug/kg/min of MR. This is illustrated in Figure 8 (Panel B) showing the percentage of the load remaining in the stomach over time. Indeed, as shown in Figure 8 (Panel C), MR completely antagonized the suppression of FER by KETO in the first 10 minutes such that FER during the administration of KETO + MR was significantly different from that during KETO alone. MR partially antagonized the effect of KETO on FER from 10 - 60 minutes such that the FER was not significantly different from control or KETO alone.

Figure 7. The effect of 0.5 ug/kg/min U50,488H (U50) and 0.1 ug/kg/min MR1452 MS (MR), alone and in combination, on gastric emptying. Panel A illustrates mean fractional emptying rate during the fasting period; Panel B, the fraction of the load remaining in the stomach over time, and Panel C, mean postload FER from 0 to 10 minutes and 10 to 60 minutes.

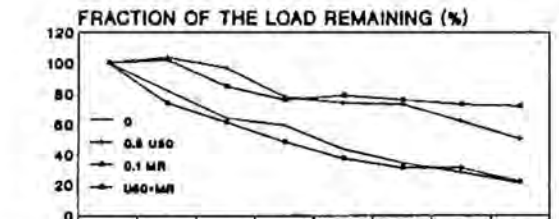
PANEL A

EFFECT OF U50,488H AND MR1452 ON FASTING FRACTIONAL EMPTYING RATE



PANEL B

EFFECT OF U50,488H AND MR1452 ON GASTRIC EMPTYING



	0	5	10	20	30	40	50	60
0	100	82	64	59	43	34	28	21
0.6 U50	100	104	97	76	74	73	62	50
0.1 MR	100	74	61	48	37	31	31	22
U50+MR	100	102	85	76	79	76	73	72

PANEL C

EFFECT OF U50,488H AND MR 1452 MS ON FRACTIONAL EMPTYING RATE OVER TIME

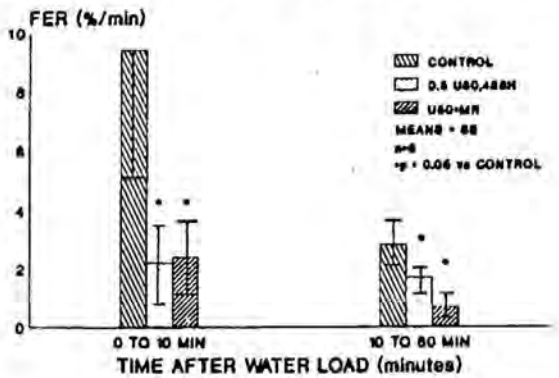
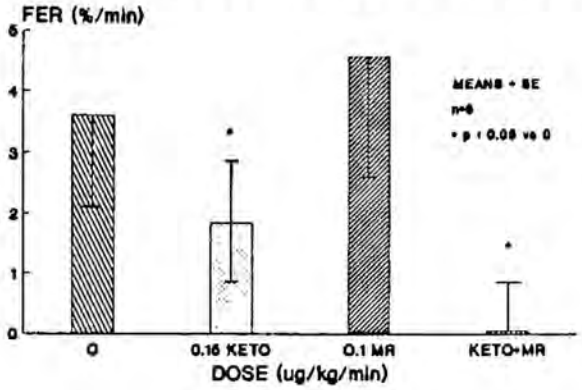


Figure 8. The effect of 0.15 ug/kg/min ketocyclazocine (KETO) and 0.1 ug/kg/min MR1452 MS (MR), alone and in combination, on gastric emptying. Panel A illustrates mean fractional emptying rate during the fasting period; Panel B, the fraction of the load remaining in the stomach over time, and Panel C, mean postload FER from 0 to 10 minutes and 10 to 60 minutes.

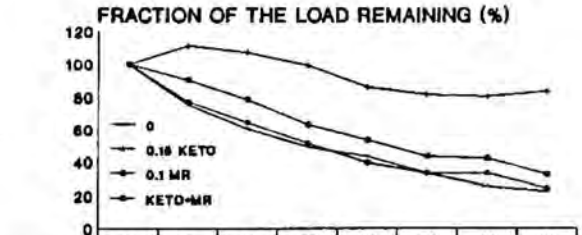
PANEL A

EFFECT OF KETOCYCLAZOCINE AND MR 1452
ON FASTING FRACTIONAL EMPTYING RATE



PANEL B

EFFECT OF KETOCYCLAZOCINE AND MR1452
ON GASTRIC EMPTYING

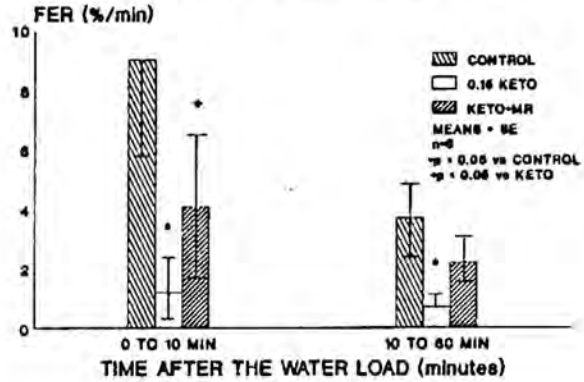


	0	5	10	20	30	40	50	60
0	100	75	60	49	43	33	25	22
0.15 KETO	100	111	107	99	86	81	80	83
0.1 MR	100	77	64	51	39	33	33	24
KETO+MR	100	90	78	63	53	43	42	32

TIME (minutes)

PANEL C

EFFECT OF KETOCYCLAZOCINE AND MR 1452 MS
ON FRACTIONAL EMPTYING RATE OVER TIME



The Effect of Kappa Receptor Agonists in Combination with MR1452 MS on Gastric Secretion

H^+ and fluid outputs were evaluted following 0.1 ug/kg/min MR given in combination with D13 (4.4 ug/kg/min), U50 (0.5 ug/kg/min), or KETO (0.15 ug/kg/min). Table 6 shows the values for gastric ion and fluid outputs for MR given with D13. When compared to control, MR given with D13 had no significant effect on H^+ or fluid outputs during the fasting period or after the load. MR completely blocked the inhibitory effect of D13 on Na^+ output during both periods. D13 alone had no significant effect on fasting or water-stimulated K^+ or Cl^- outputs or fluid outputs after the load, and these parameters were unchanged by MR + D13.

Table 3 shows that neither 0.05 nor 0.5 ug/kg/min U50 had any effect on fasting or postload fluid output. In addition, 0.5 ug/kg/min of U50 did not significantly alter ion output during either period. However, 0.05 ug/kg/min significantly increased fasting Cl^- output. These parameters remained unchanged following the administration of U50 with MR (data not presented).

Table 7 presents results obtained for fasting and water-stimulated H^+ and fluid secretion after KETO was given in combination with MR. Although the value for H^+ output following the load was not significant for 0.15 ug/kg/min of KETO alone ($p<0.10$), all other doses of KETO significantly inhibited postload H^+ output (Figure 4). This result could

Table 6. The effect of Dynorphin-(1-13) (D13: 4.4 ug/kg/min) and MR1452 MS (0.1 ug/kg/min) given alone and in combination on gastric H^+, Na^+, K^+, Cl^- and fluid outputs. Values are means ± SE; n = 5 (H^+ output, n = 6). *p<0.05 vs control; # p<0.05, vs D13 using a three-factor analysis (treatment, time, and monkey) of variance with repeated measures on the last two factors.

Table 6

	s.c. Infusion (ug/kg/min)	H^+ output (uEq/min)	Na^+ output (uEq/min)	K^+ output (uEq/min)	Cl^- output (uEq/min)	Fluid output (ml/min)
Fasting	0	7.8 ± 3.8	12.8 ± 1.9	2.8 ± 0.50	27.5 ± 5.9	0.15 ± 0.02
	4.4 D13	2.8 ± 2.6	7.8 ± 1.4*	2.0 ± 0.40	18.6 ± 1.6	0.09 ± 0.20
	0.10 MR	4.1 ± 2.5	17.2 ± 3.1	3.9 ± 0.90	30.6 ± 5.2	0.17 ± 0.04
	MR + D13	1.2 ± 1.5	12.3 ± 1.8#	2.4 ± 0.01	24.9 ± 3.5	0.15 ± 0.05
Postload	0	12.4 ± 5.3	21.4 ± 4.8	4.5 ± 0.6	32.0 ± 5.1	0.25 ± 0.40
	4.4 D13	10.9 ± 4.5	8.8 ± 0.7*	3.4 ± 0.3	29.4 ± 2.2	0.16 ± 0.03
	0.10 MR	10.5 ± 5.1	18.6 ± 1.4	4.8 ± 0.5	30.4 ± 2.2	0.23 ± 0.03
	MR + D13	6.5 ± 5.1	20.5 ± 4.1#	3.4 ± 0.2	40.7 ± 10.6	0.21 ± 0.03

Table 7. The effect of ketocyclazocine (KETO: 0.15 ug/kg/min) and MR1452 MS (MR: 0.1 ug/kg/min) given alone and in combination on gastric H^+, and fluid outputs. Values are means $\pm$ SE; n = 6. *p<0.05 or **p<0.01 vs control; # p<0.05 vs KETO using a three-factor analysis (treatment, time, and monkey) of variance with repeated measures on the last two factors.

Table 7

	s.c. Infusion (ug/kg/min)	H^+ output (uEq/min)	Fluid output (ml/min)
Fasting	0	7.8 ± 3.8	0.15 ± 0.03
	0.15 KETO	7.9 ± 4.9	0.18 ± 0.04
	0.10 MR	4.1 ± 2.5	0.20 ± 0.04
	KETO + MR	3.2 ± 2.1	0.14 ± 0.01
Postload	0	12.4 ± 5.3	0.31 ± 0.08
	0.15 KETO	6.1 ± 3.7	0.10 ± 0.08**
	0.10 MR	10.5 ± 5.1	0.19 ± 0.10
	KETO + MR	7.4 ± 2.8	0.26 ± 0.04#

be attributed to experimental variation during the 0.15 ug/kg/min dose. MR completely antagonized the suppressive action of KETO on postload fluid output. Neither fasting nor postload Na^+, K^+ or Cl^- outputs were significantly modified by 0.15 ug/kg/min KETO (Table 4) and were not altered further by MR + KETO. Thus, these data are not shown in tabular form.

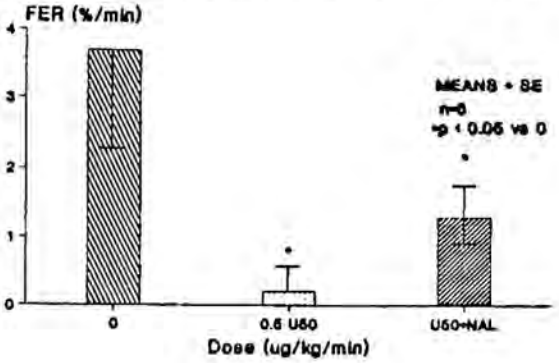
The Effect of U50,488H or Ketocyclazocine in Combination with a Non-specific Opiate Antagonist, Naloxone, on Gastric Emptying

It is generally recognized that the way to demonstrate that an agonist is exerting its action by acting at an opioid receptor is to inhibit these effects by blocking the receptors with NAL, a non-specific opiate antagonist. Studies utilizing NAL alone were not carried out as a part of this research. Previous studies in our laboratory showed that it has no effect on gastric FER or fluid, acid, or electrolyte secretion in primates at the dose given in these studies (40 ug/kg bolus of NAL followed by 4 ug/kg/min NAL) (Shea-Donohue et al., 1983). Naloxone was administered in combination with U50 (0.5 ug/kg/min) or KETO (0.15 ug/kg/min). Figure 9 presents the effect of U50 given in combination with NAL on mean FER during the fasting period (Panel A). NAL was unable to overcome the inhibitory effect of U50 on FER during the fasting period. In addition, NAL

Figure 9. The effect of 0.5 ug/kg/min U50,488H (U50) alone and in combination with naloxone (NAL; 40 ug/kg bolus i.v. followed by 4 ug/kg/min s.c.) on gastric emptying. Panel A illustrates the mean fractional emptying rate (FER) during the fasting period; Panel B, the fraction of the load remaining in the stomach over time, and Panel C, mean postload FER from 0 to 10 and from 10 to 60 minutes.

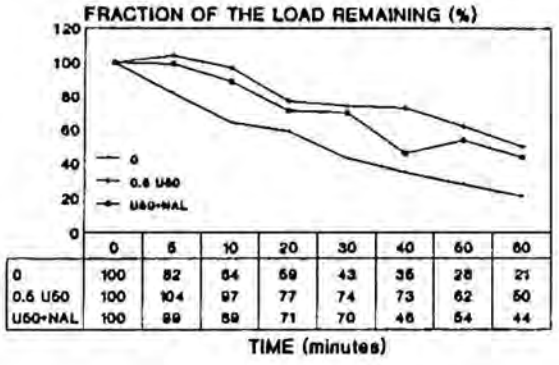
PANEL A

EFFECT OF U50,488H AND NALOXONE ON FASTING FRACTIONAL EMPTYING RATE



PANEL B

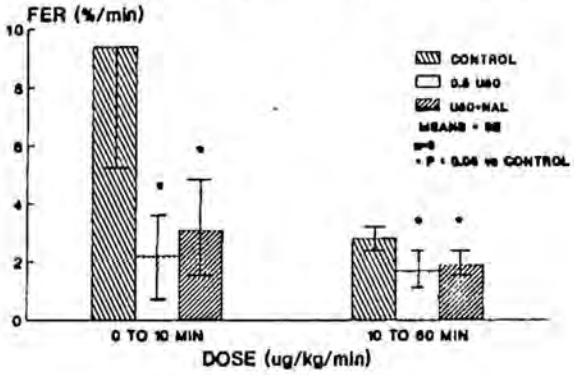
EFFECT OF U50,488H AND NALOXONE ON GASTRIC EMPTYING



	0	5	10	20	30	40	50	60
0	100	82	64	59	43	35	28	21
0.5 U50	100	104	97	77	74	73	62	50
U50+NAL	100	89	59	71	70	46	54	44

PANEL C

EFFECT OF U50,488H AND NALOXONE ON FRACTIONAL EMPTYING RATE OVER TIME



did not antagonize the U50-induced delay in gastric emptying throughout the entire period after the water load (Figure 9, Panels B and C).

The results for KETO given with NAL on fasting and postload FER are shown in Figure 10. NAL was able to partially antagonize the inhibitory action of KETO on fasting FER such that KETO + NAL was not significantly different from control (72% of control value) or from KETO alone (Panel A). Figure 10 (Panel B) demonstrates the effect of KETO + NAL on the fraction of the load remaining in the stomach over time. These data show that NAL antagonized the inhibitory action of KETO on gastric emptying. When the FER following the intragastric administration of water was evaluated from 0 to 10 minutes and from 10 to 60 minutes, it was found that the suppression of FER by KETO was prevented significantly by NAL during the early phase (0-10 min) of gastric emptying. From 10 to 60 minutes, NAL partially antagonized the effect of KETO such that FER during KETO + NAL was not different from either control or KETO + NAL (KETO + NAL = 60% of the control value).

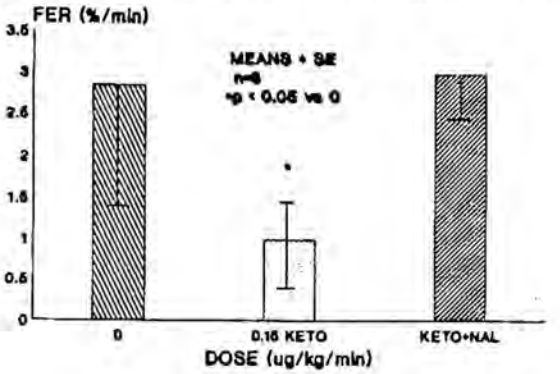
The Effect of U50,488H or Ketocyclazocine in Combination with Naloxone on Gastric Secretion

The effects of U50 (0.5 ug/kg/min) administered together with NAL (40 ug/kg bolus NAL followed by 4

Figure 10. The effect of 0.15 ug/kg/min ketocyclazocine (KETO) alone and in combination with naloxone (NAL: 40 ug/kg bolus i.v. followed by 4 ug/kg/min s.c.) on gastric emptying. Panel A illustrates the mean fractional emptying rate (FER) during the fasting period; Panel B, the fraction of the load remaining in the stomach over time, and Panel C, the mean postload FER from 0 to 10 minutes and 10 to 60 minutes.

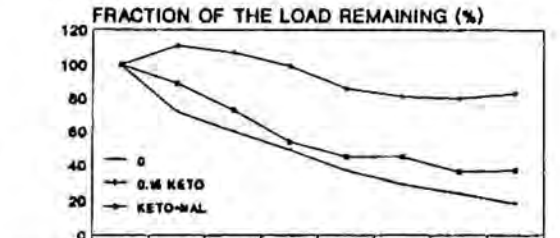
PANEL A

EFFECT OF KETOCYCLAZOCINE AND NALOXONE ON FASTING FRACTIONAL EMPTYING RATE



PANEL B

EFFECT OF KETOCYCLAZOCINE AND NALOXONE ON GASTRIC EMPTYING

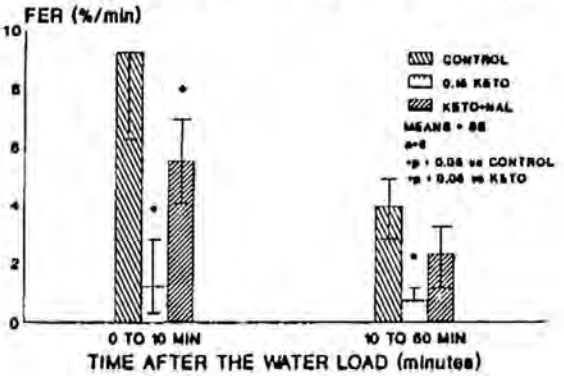


	0	5	10	20	30	40	50	60
0	100	72	60	49	37	29	24	18
0.16 KETO	100	111	107	99	86	81	80	83
KETO+NAL	100	89	73	64	45	45	36	37

TIME (minutes)

PANEL C

EFFECT OF KETOCYCLAZOCINE AND NALOXONE ON FRACTIONAL EMPTYING RATE OVER TIME



ug/kg/min NAL) on fasting and water load-stimulated ion and fluid outputs are presented in Table 8. While U50 or NAL given alone had no significant effect on these parameters, the drugs given in combination significantly increased fluid outputs during both periods, and the fasting value was significantly higher than either U50 alone or that of control. When U50 was administered with naloxone, the fasting Cl^- and postload K^+ outputs were enhanced significantly when compared to control.

Table 9 illustrates the effect of KETO given with NAL on fasting and postload ion and fluid outputs. NAL did not modify the inhibitory effect of KETO on H^+ output. In contrast, following KETO + NAL, both fasting and water load-stimulated fluid outputs were significantly increased above KETO, and the fasting fluid value was significantly greater than control. The postload value for Na^+ following KETO + NAL was significantly greater than that of KETO. K^+ output was increased significantly by KETO + NAL during both periods when compared to either control or KETO alone.

Table 8. The effect of U50,488H (U50: 0.5 ug/kg/min) alone and in combination with Naloxone (NAL: 40 ug/kg bolus plus 4.0 ug/kg/min) on gastric acid H^+, Na^+, K^+, Cl^- and fluid outputs. Values are means $\pm$ SE; n = 6. *p<0.05 or **p<0.01 vs control and # p<0.05 vs U50 using a three-factor analysis (treatment, time, and monkey) of variance with repeated measures on the last two factors.

Table 8

	s.c. Infusion (ug/kg/min)	H^+ output (uEq/min)	Na^+ output (uEq/min)	K^+ output (uEq/min)	Cl^- output (uEq/min)	Fluid output (ml/min)
Fasting	0	1.8 ± 1.8	17.3 ± 3.8	6.7 ± 0.7	14.9 ± 1.1	0.15 ± 0.02
	0.5 U50	2.6 ± 1.6	14.3 ± 2.9	4.3 ± 0.6	22.4 ± 1.6	0.14 ± 0.02
	U50 + NAL	0.6 ± 0.5	19.6 ± 3.3	8.4 ± 1.0	38.6 ± 4.2*	0.26 ± 0.04**#
Postload	0	3.8 ± 0.15	16.9 ± 2.3	4.0 ± 0.2	26.1 ± 2.2	0.25 ± 0.04
	0.5 U50	3.4 ± 2.20	26.9 ± 2.1	5.6 ± 0.3	36.6 ± 2.9	0.30 ± 0.06
	U50 + NAL	4.1 ± 2.10	21.2 ± 1.8	9.2 ± 0.9*	38.3 ± 2.3	0.48 ± 0.14**

Table 9. The effect of ketocyclazocine (KETO; 0.15 ug/kg/min) given alone and in combination with Naloxone (NAL: 40 ug/kg bolus plus 4.0 ug/kg/min) on gastric H^+, Na^+, K^+, Cl^- and fluid outputs. Values are means ± SE; n = 5 (H^+ output, n = 6). *p<0.05 or **p<0.01 vs control and #p<0.05 vs KETO using a three-factor analysis (treatment, time, and monkey) of variance with repeated measures on the last two factors.

Table 9

	s.c. Infusion (ug/kg/min)	H^+ output (uEq/min)	Na^+ output (uEq/min)	K^+ output (uEq/min)	Cl^- output (uEq/min)	Fluid output (ml/min)
Fasting	0	7.5 ± 3.2	13.8 ± 2.5	2.9 ± 0.4	24.5 ± 3.5	0.15 ± 0.03
	0.15 KETO	7.9 ± 4.9	19.6 ± 2.2	3.6 ± 1.6	16.5 ± 3.3	0.18 ± 0.04
	KETO + NAL	2.7 ± 2.7	24.5 ± 5.4	11.7 ± 1.4**#	33.5 ± 7.6	0.35 ± 0.05*#
Postload	0	13.5 ± 5.2	21.5 ± 7.5	4.0 ± 0.7	36.3 ± 4.5	0.40 ± 0.06
	0.15 KETO	6.1 ± 3.7	11.3 ± 0.3	2.3 ± 0.2	27.8 ± 3.0	0.10 ± 0.08*
	KETO + NAL	4.5 ± 4.5	28.9 ± 5.4#	11.1 ± 1.7**#	40.5 ± 4.3	0.50 ± 0.02#

DISCUSSION

These studies were designed to evaluate the role of kappa receptor agonists and antagonists on gastric emptying and secretion in primates. The results demonstrate that kappa receptors may play a role in the peripheral control of gastric emptying and suggest that they also affect non-parietal but not parietal secretion. These data will be considered separately.

A primate model was utilized because monkeys have a close, phylogenetic relationship to humans. The animals were conscious as anaesthesia is known to modify gastric function and opiates are known to have opposite effects in anaesthetized vs unanaesthetized animals (Saunders et al., 1987). Opiate agonists and antagonists were administered alone and in combination as subcutaneous infusions. Thus, the effects of these drugs were roughly analogous to those of a circulating hormone. One advantage of this route of adminstration is that opiates with a short half-life (<10 seconds) such as dynorphin-(1-13) are continuously put into the circulation, increasing the probabilty that they will produce an effect prior to enzymatic degradation. In addition, gastric samples were taken every ten minutes during a fasting period as well as following the administration of a water load. Multiple sampling allowed the measurement of concurrent changes in gastric FER and fluid and electrolyte secretion during the fasting and in the stimulated states.

Thus, physiological changes in the compositions of the gastric secretions under two conditions were evaluated.

A dye-dilution technique was used to measure gastric emptying, secretion and intragastric volume simultaneously. It is important to measure secretion and emptying concurrently as the intragastric H^+ concentration plays a major role in the regulation of gastric emptying. Another advantage of this technique is that it allows the experimentalist to follow changes in emptying and secretion due to factors such as duodenal feedback to proceed physiologically.

An important difference between our work and most studies involving kappa agonists is the magnitude of the dosage used and how it was given. In our work, the cumulative dose of any agonist was less than one mg (D13: 0.3-0.5 mg/kg; KETO 0.01-0.1 mg/kg; U50: 0.006-0.6 mg/kg), and was infused continuously. In experiments carried out in rats or mice to evaluate gastrointestinal motility and acid secretion, other workers routinely used higher doses of opiates in bolus amounts than we utilized in our studies (dynorphin-(1-9): 1-10 mg/kg; ketocyclazocine: 0.3-3.0 mg/kg; U50,488H: 1-100 mg/kg) (Porreca et al., 1983; Shook et al., 1987; Fox and Burks, 1988). This suggests that our model (primate) is more sensitive to opiates than other workers' (rats or mice) and differences between our results and those of other workers may be attributed to many factors that determine

sensitivity to a a particular drug. These variables include the following: 1) a drug's affinity to a receptor and its selectivity, 2) its accessibility to receptors, 3) its stability in the circulation, 4) its biological response, 5) its central effects and 6) variations in opiate receptor subpopulations among species.

Gastric Motility

The kappa receptor agonists, dynorphin-(1-13), U50,488H and ketocyclazocine were demonstrated to be inhibitors of gastric emptying in primates. An inhibitory effect was also repeated in the same system utilizing other opioid agonists. Both the delta receptor agonist, met-enkephalin and its synthetic analogue, [D-Ala^2]Met-enkephalinamide (DMET) (Shea-Donohue et al., 1983) and the mu agonist, morphine, (Feldman et al., 1980; Feldman and Cowley, 1982; Bianchi et al., 1982; Porreca et al., 1983) slow gastrointestinal transit in a variety of species.

Taken as a group, the inhibition of fasting gastric emptying by kappa agonists in our studies ranged from 56-92% of control (Figures 1, 2 and 3), with KETO giving the lowest and highest degree of inhibition. The cumulative number of moles of the highest dose (1.0 ug/kg/min) of KETO (1.1×10^{-7} moles) was lower than either the 4.4 ug/kg/min dose of D13 (1.9×10^{-6} moles) or 0.5 (5.0×10^{-7} moles) and 5.0 ug/kg/min (5.3×10^{-6} moles) dose of U50. If the kappa

agonists act at the same subtypes of receptors and if the attractive forces between the opiates for their receptors are identical, the highest inhibition by KETO may have been due to a longer half-life than that possessed by either D13 or U50. Inhibition by the low doses of KETO could have resulted from its activity at a high affinity mu or kappa site. Alternatively, the suppressive effect could reflect KETO's ability to cross the blood-brain barrier and produce centrally-mediated inhibition. An added factor to be considered is that recent studies have demonstrated that kappa receptor antagonists that are very potent in vitro may not be as effective in vivo (Birch et al., 1987) because it is difficult in vivo to control or to measure accurately the actual concentration of the drug available to the receptors. In addition, drugs that are given subcutaneously or intravenously are distributed to all the organ systems of the body, and the drugs may induce responses in these systems which may or may not affect the experimental results of the actual system being studied. Finally, ketocyclazocine's effects on fasting gastric emptying and secretion may, in part, not be mediated via an opiate receptor.

All kappa agonists reduced gastric emptying following the administration of a water load. One way to evaluate this reduction was to determine the percentage of the load remaining in the stomach over time. Viewed in this fashion, the high dose of D13, the two highest doses of U50, and all

doses of KETO increased the percentage of the load remaining in the stomach throughout the entire period (Figures 1, 2, 3: Panel B). When the gastric fractional emptying rate was determined following the intragastric instillation of water and divided arbitrarily into two periods: an early phase (0 -10 min) and a late phase (10 - 60 min), all of the agonists studied inhibited emptying during the entire time. However, the relative effect was greater during the early phase (0- 10 min) than in the later phase (10- 60 min) (Figures 1-3). Together, these results suggest that kappa receptor agonists may play a physiological role in response to a stimulus such as distention. Gastric emptying of liquids is known to be regulated primarily by the fundus and mediated by acetylcholine released from enteric neurons. Thus, kappa agonists could be suppressing fundic contraction and inhibiting emptying by reducing the release of acetylcholine from those neurons. Therefore, if dynorphin were released by gastric distention in a fashion similar to that observed in the distention of the ileum (Donnerer et al., 1984), endogenous kappa agonists may act as ongoing modulators of gastric emptying by negative feedback.

To further investigate the hypothesis that endogenous ligands of kappa receptors regulate gastric function, the putative kappa receptor antagonist, MR1452 MS, was administered and its effect on gastric emptying was evaluated. MR1452 MS was a gift from Boerhinger-Engelheim and

was reported to be the most selective kappa antagonist available. When compared to control, low doses (0.05 or 0.1 ug/kg/min) of MR1452 MS had no significant effect on the fasting mean fractional emptying rate. Thus, endogenous kappa receptor ligands may not influence emptying in the fasting state. However, during the first ten minutes following the instillation of water, the high dose (0.25 ug/kg/min) of MR1452 MS given alone inhibited the early phase of gastric emptying (Figure 5: Panel C). As exogenous kappa agonists also inhibited FER during this time, the suppressive effect of MR suggests that, at high doses, this antagonist acts as a partial agonist. Since our data suggest that MR is a partial agonist, this result could also explain why MR did not affect fasting FER.

It is generally recognized that the way to show that an observed effect of an opioid is mediated by a particular type of opiate receptor is to demonstrate its suppression by a selective opiate antagonist. To test this hypothesis, MR was given in combination with D13, U50 or KETO. As shown in Figure 5, the dose of MR chosen to be given with the drugs in our studies (0.1 ug/kg/min) was the highest dose that could be used without its acting as an agonist by itself. MR was unable to overcome the inhibitory effects on emptying during the fasting period for any agonist. In addition to the factors listed above for a drug's not producing an effect, it is possible that MR was given in a dose too low

to affect these drugs; fasting gastric emptying may not be opioid mediated or MR may not be acting at a kappa receptor. In contrast, following gastric distention induced by a water load, the effect of MR was variable. MR significantly antagonized D13's inhibition of gastric emptying during the second phase of the postload period (10 - 60 min), but was ineffective against U50,488H during either period. The inhibition of gastric emptying induced by KETO was completely blocked during the first ten minutes following the water load by MR, but was only partially antagonized by MR during the later postload (10 - 60 min) period. A reasonable explanation for the different responses of D13 in the presence of MR following the water load could be that MR was being broken down enzymatically in the first hour of the study (during fasting and first ten minutes postload) and that by 10-60 minutes, a sufficient percentage of MR could have escaped enzymatic degradation and accumulated for it to exert its blocking effect. Since MR had no effect on U50's suppression of emptying, it is possible that D13 and U50 bind to separate subtypes of kappa receptors to which MR had no affinity. Alternatively, since U50 was administered in molar amounts significantly greater (5.0×10^{-6} moles) than was MR (1.1×10^{-7} moles), MR may not have been able to inhibit competetively U50's inhibition of gastric emptying. Finally, MR could have antagonized the kappa but not the mu effect of ketocyclazocine.

The present studies on kappa receptor function in primates are in contrast to those recently carried out on studies of opiates on gastrointestinal transit in rats by Shook et al. (1987). They showed that the selective, synthetic kappa receptor ligand U50,488H had no effect on gastrointestinal motility when administered peripherally. These differences from our experiments may be due to several factors. First, they utilized higher doses (100 mg/kg s.c.) which were administered as bolus injections. We used a continuous, subcutaneous infusion (0.05, 0.5 or 5.0 ug/kg/min U50 s.c.). Secondly, the duration of their experiments was 35 minutes, much shorter than ours (100 minutes). Thirdly, they used rats and we used primates. Finally, it has been demonstrated that there are at least two separate kappa receptor subtypes in humans, rats and guinea pigs (Pfeiffer et al., 1981; Quirion et al., 1982; 1987; Bunn and Wilkin, 1988; Zukin et al., 1988). Thus, differences may also be attributed to a different distribution of receptors mediating gastric function in the two species. Therefore, unlike the rat, primates may have a peripheral kappa receptor subpopulation capable of mediating gastric function, while rats do not.

The lack of an effect of MR on U50's suppression of motility and MR's partial antagonism of ketocyclazocine on gastric emptying in primates was further evaluated using naloxone, a non-specific opioid antagonist. It is generally

recognized that it requires a ten-fold higher dose of naloxone to block kappa than delta or mu receptors. The dose of naloxone selected had been shown to have no effect on gastric function but to block the actions of delta receptor agonists in primates (Shea-Donohue et al., 1983). As shown in Figure 9, naloxone was unable to overcome the U50-induced delay in gastric emptying during the fasting period or following the water load. Assuming U50's effect is mediated through the kappa receptor, these results suggest that this dose of naloxone is unable to antagonize the kappa receptor. Alternatively, since naloxone was unable to antagonize the U50's inhibition of emptying, it allows the possibility that U50's effect on emptying is modulated by a kappa receptor subtype other than the one at which D13 is active or is a non-opiate mediated effect. In contrast, NAL completely overcame the inhibition of gastric emptying induced by KETO during the fasting period; completely prevented the inhibition of gastric emptying in the early period following the water load, and partially inhibited the effect of KETO during the later period (10 - 60 min) (Figure 10: Panel C). Since NAL is known to have greater affinity for mu than kappa receptors, the inhibitory effect of KETO on the gastric emptying could be due, in large part, to its activity at the mu receptor, and suggests that KETO acts at mu receptors.

While this work was being revised for publication, a report appeared which confirms our findings with respect to the gastric emptying of water. Gastric liquid emptying in canines was inhibited by the selective kappa agonists, U50,488H (0.1 mg/kg p.o.) and tifluadom (0.1 mg/kg p.o.) (Gue et al., 1988). Since they administered the opiates orally, their research is particularly interesting because the results support the idea that kappa opioid peptides and agonists modulate gastric emptying by activating receptors in the gastric mucosa and submucosa. In addition, by using MR 2266 (0.1 mg/kg i.v.) a different, reportedly potent selective kappa receptor antagonist (Magnan et al., 1982) than the ones used in our studies, Gue and associates were able to antagonize U50's inhibition of gastric emptying. These studies suggest that liquid gastric emptying is mediated by kappa receptors. Since duodenal motility after feeding in dogs was unaltered in response to the same doses of orally administered kappa agonists, they concluded that kappa receptor agonists enhance the gastric relaxation stimulated by feeding. The final mechanism of this delayed emptying is unknown and may be due to pyloric constriction, reduced contractile strength of fundic smooth muscle, or both. These effects could be the results of neural mechanisms which are centered in the brain, and mediated via the vagus. Alternatively, they could also be regulated locally by receptors located on the circular smooth muscle, on the

enteric neurons, or on the gastroduodenal mucosa or submucosa. Gastric secretion in dogs apparently is not affected by U50,488H and other kappa receptor agonists (Bartolini et al., 1985) and Gue and his group made no attempt to measure it. The fact, however, that kappa receptor agonists did alter fluid and electrolyte secretion in our studies suggests that there are species differences occurring in kappa receptor subpopulations.

Although it may be possible that the effects of the kappa agonists in this present work were produced by actions on the brain or spinal cord, it seems most likely that our results were produced by a direct effect of the agonists on the stomach. Several facts support this interpretation. Small peptides such as dynorphin-(1-13) do not cross the blood-brain barrier because of their state of ionization (Cornford et al., 1978). In addition, no detectable respiratory or behavioral changes in the monkeys were observed in our studies. However, large doses of U50,488H and KETO have been reported to decrease respiratory activity and to produce sedation and analgesia in primates via a centrally-mediated action (Dykstra et al., 1987). Furthermore, ketocyclazocine has been shown to inhibit gastrointestinal transit only when it was administered subcutaneously, not when given into the cerebral ventricles (Porreca et al., 1983).

If the effect of the kappa agonists were directly on the stomach, several factors should be considered in an explanation of how emptying was depressed. An increase in the local release of a smooth muscle relaxer such as vaso-active intestinal peptide (VIP) could certainly contribute to the inhibition of gastric emptying. However, this does not seem likely since kappa agonists are reported to inhibit, not increase, the release of VIP from the small intestine in dogs (Huidoboro-Toro et al., 1988). As mentioned above, gastric emptying could be reduced if the pylorus offered more than the usual resistance to outflow of chyme. Allescher and co-workers (1988) found that intra-arterially infused dynorphin-(1-13) inhibited continuous contraction of electrically-stimulated pyloric muscle in the dog. In the same study, dynorphin-(1-13) also reduced the spontaneous activity of pyloric muscle. The results of Allescher's work indicate that kappa agonists relax the pylorus, not contract it. Thus, it seems probable that dynorphin would likely have a similar inhibiting effect on the contractions of the rest of the stomach.

Opioid agonists are known to inhibit the release of acetylcholine from myenteric neurons (Kromer and Schmidt, 1982). Such an action could operate to reduce contraction of stomach muscle and, therefore, emptying. Both Paton (1957) and Schaumann (1957) reported that opioids directly depressed acetylcholine release from myenteric neurons. In

a somewhat similar study, Goldstein and associates (1979) found that opioid agonists inhibited electrically-induced contractions of guinea pig ileum strips. Such effects in vivo would, of course, lead to the reduction in the release of acetylcholine. Thus, kappa receptor agonists may inhibit gastric emptying by directly activating kappa receptors in the myenteric plexus.

Bitar and Mahklouf (1985) have incorporated these facts in a model, which, although aimed at opiate actions in the intestine, may apply to their inhibitory effects on the neuromuscular apparatus of the stomach. This system has two direct routes (neural and muscular) and one indirect route (neural) whereby opiates could modulate propulsive activity in the gastrointestinal tract. They suggest that the direct pathway affects the presynaptic release of acetylcholine from myenteric neurons or directly activates opiate receptors on circular smooth muscle cells themselves. The indirect route is proposed to involve the alteration of the activity of inhibitory interneurons in the myenteric plexus. By applying such a model to the stomach, a stimulus such as distention, could result in an increase in the release of endogenous opiates. These, in turn, could reduce the release of acetylcholine and modulate the activity of the myenteric neurons which normally stimulate the smooth muscle cells (especially circular). As a result, emptying of the stomach would be reduced.

From the above considerations, it seems reasonable to exclude an increase in the resistance of the pyloric sphincter and/or an increase in the local release of VIP as the reason for the decrease in gastric emptying demonstrated in our studies. What seems most likely is that dynorphin-(1-13), U50,488H and ketocyclazocine inhibited the pre-synaptic release of acetylcholine from nerve endings. However, a direct, suppressive effect on smooth muscle cells cannot be excluded.

Gastric Secretion

As with other physiological secretions, the ions of gastric juice are secreted first and water follows osmotically. This mixture is the largest part of the juice and its precise composition depends upon the cell type of origin (Hunt and Wan, 1967). Gastric secretion may be considered to have parietal and non-parietal components. H^+ output reflects parietal secretion and Na^+ is taken as an index of non-parietal secretion. K^+, Cl^- and fluid outputs are components of both types of secretion.

In our studies dynorphin-(1-13) did not affect parietal secretion as neither the fasting output of H^+ nor its output following the administration of water was altered significantly. However, during both periods, D13 significantly inhibited Na^+ output. Since Na^+ output is considered to be an index of non-parietal secretion, this finding

suggests that D13 suppresses non-parietal cell secretion.

MR completely antagonized D13's inhibition of fasting and postload Na^+ outputs. Since the dosage of MR was about an order of magnitude less, i.e., D13: 1.9×10^{-6} moles vs MR: 1.1×10^{-7}, it may be that MR has a higher affinity for the receptors mediating Na^+ secretion than does D13. In contrast to its effect on Na^+ secretion, MR inhibited D13's suppressive action on motility during the later phase of postload period but had no significant effect during the fasting state. If D13 and MR do have the different affinities for kappa receptors, then differences in the effect of MR + D13 on motility and secretion may be explained if kappa receptors modulating gastric emptying have a lower affinity for kappa agonists than those regulating secretion.

In contrast to D13, U50 had no significant effect on fluid or ion output during either period. The difference may be due to one or more factors. First, as suggested by their variable effects on motility, U50,488H may act at a different kappa receptor from the one at which dynorphin is active. Secondly, although equimolar doses of U50,488H and dynorphin-(1-13) were administered, a higher dose of U50 may be required to affect fluid and ion secretion than the dosage that inhibited motility. This could be the case if there are two gastric kappa receptor subtypes having different affinities. There may be a high affinity receptor

modulating motility in response to low concentrations of U50 and a low affinity site which affects secretion only in response to high concentrations of this opiate. Finally, U50,488H has been shown to be at least 100-fold more selective for kappa receptors than other known kappa agonists (Lahti et al., 1982; VonVoightlander et al., 1983), while D13 has some activity at mu and delta receptors (Gouarderes et al., 1983; James et al., 1983). Thus, the inhibitory effect of D13 on Na^+ may not occur through the kappa receptor. This last idea is supported by the fact that ketocyclazocine, a mixed kappa/mu agonist, significantly inhibited the outputs of fluid and Na^+. As expected, since U50,488H alone did not alter significantly ion or fluid output, MR given in combination with that opiate did not modify the response.

When U50,488H was administered in combination with the antagonist naloxone (Table 8), in approximately equimolar amounts (5×10^{-6} moles vs 5.9×10^{-6} moles), the output of K^+ was significantly increased following a water load and fasting Cl^- was significantly enhanced. The lack of an increase of H^+ ion casts doubt on the possibility of increased secretion from parietal cells. While the ion data does not firmly support an increase either in parietal or non-parietal secretion, fluid output was stimulated significantly. The mechanism of this action is unknown. It may be that this enhancement of fluid, K^+ and Cl^- secretion

by naloxone plus U50 is due to its blocking gastric mu receptors and permitting the events mediated by kappa receptors to occur or to be enhanced. A precedent for this idea comes from VonVoightlander's group (1983) and Leander and his coworkers, (1987) who proposed that actions mediated by U50,488H and other kappa receptor agonists are "unmasked" when naloxone is given to rats in doses which suppress mu opioid agonist activity. The mechanism of this unmasking effect could occur if the opiate receptor were a single, large molecule which expresses its mu, delta and kappa subtypes by changing its conformation (Bowen et al., 1981). Thus, for example, NAL's binding to the mu site could expose a kappa receptor subtype which had previously been unavailable to U50. This could account for the increases in fluid and electrolyte secretion when U50 was administered in combination with naloxone. In addition, the potentiation observed in these parameters when NAL was given with KETO could be explained by a similar mechanism.

Our results show that KETO suppressed acid secretion, suggesting that its ultimate effect may be directly on the parietal cell. Although the mechanism by which ketocyclazocine affects acid secretion is uncertain, plasma gastrin levels remained unchanged in these studies, indicating that KETO's inhibition of acid secretion was not due to a decrease in circulating gastrin. Since ketocyclazocine is known to be active at both mu and kappa receptors, KETO's

effect on acid secretion in our work may be mediated primarily by the mu receptor. This result is similar to previous findings in which the mu receptor agonist, morphine, altered gastric acid secretion but had no effect on plasma gastrin (Olson et al., 1982).

In order to determine if the suppression of acid secretion by KETO was mediated in part by kappa receptors, KETO (0.15 ug/kg/min) was given in combination with MR (Table 7). MR significantly overcame KETO's inhibition of postload fluid output. KETO, when administered alone (0.15 ug/kg/min), produced no significant change in acid secretion. This was surprising, since in our preliminary studies, the doses immediately above and below were found to significantly inhibit acid secretion. Therefore, because MR was given in combination with the 0.15 ug/kg/min dose, it was not possible to ascertain if MR could have antagonized the inhibition of acid secretion produced by the other doses. Since MR antagonized completely the decrease in Na^+ output induced by D13 and blocked KETO's inhibition of fluid output, it seems likely that D13, KETO and MR are mediating non-parietal secretion by the same kappa opiate receptor. In addition, NAL significantly potentiated fasting and postload K^+ outputs and fasting fluid output. Such potentiation could occur if NAL were blocking the mu receptors and permitting the expression of kappa mediated effects to

occur or if NAL were acting synergistically with KETO at the same receptor to increase non-parietal secretion.

MR when given alone significantly enhanced fluid and Cl^- output in the absence of any significant effect on acid secretion. However, Cl^- is secreted by both types of cells. Since the secretion of H^+ and Na^+ were unaltered by MR, we are unable to discern from these data whether MR was altering parietal or non-parietal secretion. This could be evaluated if higher doses of MR altered acid or Na^+ output.

In general, secretion was less depressed by the agonists than was motility. Such suppression as did occur could have been produced by the same overall reduction in the amount of acetylcholine available to secretory cells. Based on the reduced output of Na^+, D13 decreased non-parietal secretion but had no significant effect on parietal secretion (Table 2), while ketocyclazocine suppressed both (Table 4). MR blocked the inhibition of non-parietal secretion produced by D13 (Table 6), which suggests that they could be acting at the same kappa receptor. Since U50 had no effect on either type of secretion (Table 3), while D13 did inhibit non-parietal secretion, there may be at least two kappa receptor subtypes present in the stomach. The inability of MR to antagonize U50 and the fact that naloxone given in combination with U50 increased fluid secretion suggests that MR may not be a kappa receptor antagonist or, more likely, may not act at the same kappa receptor as U50

at the doses administered. This idea correlates with our studies of gastric motility in which MR was unable to antagonize U50's inhibition of emptying.

It has been reported that kappa receptor agonists produce diuresis in rats under normal, water-loaded and water deprived conditions, and naltrexone, a peripheral opiate antagonist with properties similar to those of naloxone, enhances this effect (Leander et al., 1987). It is interesting that in our work, as in Leander's, U50 or KETO given in combination with naloxone increased fluid output suggesting they act at the same receptor subtype. This could have occurred if naloxone at the dose used (40 ug/kg bolus plus 4 ug/kg/min) blocked primarily mu and/or delta receptors. Such selective antagonism would allow the action of the kappa receptor to become dominant. This suggests that endogenous kappa agonists increase fluid secretion, an action which may be modulated by mu activity. Since kappa-selective agonists did not modify significantly acid secretion, this fluid may be part of non-parietal secretion. Therefore, these results indicate that the inhibition of non-parietal secretion produced by D13 may not be mediated by the kappa receptor, although D13 may be acting at a receptor subtype other than the one at which U50 is active. It is important to note, however, that neither D13 nor U50 significantly affected acid secretion, while delta and mu agonists do alter acid secretion. This fact is

evidence of a functional specificity among opioid receptor subtypes.

SUMMARY AND CONCLUSIONS

The effects of kappa receptor agonists and antagonists on gastric emptying and secretion, fluid and ion outputs were evaluated in conscious chair-adapted rhesus monkeys. The animals were studied during a fasting period and following the administration of an 80 ml water load (postload, mild distension stimulus). A dye-dilution technique was used to determine concurrently fractional emptying rate (FER = ml emptied/intragastric volume), fluid and ion output. The results can be summarized as follows:

SUMMARY

Gastric Emptying:

Dynorphin-(1-13), an endogenous ligand of the kappa receptor, significantly inhibited the mean gastric fractional emptying rate during the fasting period. In addition, it delayed gastric emptying of the water load during the entire 60 minute period. The putative, highly selective kappa receptor antagonist, MR1452 MS, did not significantly modify gastric function at lower doses but at higher doses had a significant inhibitory action on gastric emptying after the intragastric administration of water. Following the water load, MR significantly antagonized the inhibitory effect of dynorphin-(1-13) during the late phase (10 - 60 min). U50,488H, a selective, synthetic kappa

receptor agonist, significantly inhibited the mean gastric fractional emptying rate during the fasting and postload periods. Neither MR nor naloxone, a non-specific opiate antagonist, at doses having no significant effects on gastric function, were able to block the suppressive effect of U50 during either period.

Ketocyclazocine, an opiate agonist with both kappa and mu receptor activity, suppressed mean fasting fractional emptying rates. This opiate agonist also produced decreases in emptying following the administration of the water load, which resulted in a significant increase in the percentage of the load remaining in the stomach during the entire postload period. Neither MR nor NAL in combination with KETO had any effect on fasting gastric emptying. Following the water load, both MR and NAL significantly inhibited KETO's reduction of gastric emptying during the early phase (0 - 10 min) and NAL prevented the reduction in gastric emptying induced by KETO during the first ten minutes following the water load and attenuated KETO's effect during the later phase (10 - 60 min).

Acid and Fluid Outputs

D13 had no effect on H+ output during either the fasting or following the water load. As in the case of D13, U50 did not alter fasting H^+ or fluid output and did not affect either parameter following the administration of a

water load. However, NAL + U50 significantly increased fluid above that of control. Fluid output was significantly inhibited by the high dose of KETO during the fasting period and by all doses of KETO following the water load. Fasting and postload H^+ outputs were also significantly decreased by KETO. Plasma gastrin levels were unchanged by KETO. MR and NAL completely blocked the suppressive action of KETO on fluid output after the load, but did not affect H^+ output during either period. In addition, NAL + KETO significantly increased fluid above control.

Sodium, Potassium and Chloride Outputs

D13 significantly inhibited both fasting and water load-stimulated Na^+ outputs, but had no effect on K^+ and Cl^- outputs. MR blocked the suppression of Na^+ output induced by D13 during both periods. The lowest dosage of U50 significantly increased fasting Cl^- output but did not significantly alter Na^+, K^+ or Cl^- outputs after the water load. However, NAL in combination with U50 significantly enhanced the fasting Cl^- and postload K^+ value from that of control. KETO significantly suppressed Na^+ output during the fasting period and following the water load, and KETO also significantly decreased Na^+ and K^+ after the intragastric administration of water. Naloxone administered in combination with KETO significantly potentiated fasting K^+ secretion above

that of control and significantly increased Na^+ and K^+ output above KETO alone after the water load.

CONCLUSIONS

All the kappa agonists studied inhibited gastric emptying during a fasting period and after the intragastric administration of water. This inhibition is most prominent during the early phase (0 - 10 min) following the administration of a water load and is similar to that observed with mu and delta agonists by other workers. The mechanism of this action is unknown. However, such an effect might occur if the inhibition of gastric emptying in response to distention were mediated cholinergically by kappa receptor agonists. Thus, if the greatest percentage of endogenous opiates were released in response to distention within the first ten minutes and that percentage is decreased in the subsequent 10 to 60 minutes, the response would diminish after the initial ten minutes.

Selective kappa receptor agonists, D13 and U50 had no significant effect on acid secretion either during the fasting period or following the water load. However, KETO, which is known to have selectivity for mu and kappa receptors, decreased acid secretion during both periods. Since earlier work has shown that mu and delta receptor agonists alter acid secretion, it seems likely that this effect of KETO was mediated by stimulation of mu or delta receptors

and may reflect a functional specificity for acid secretion among opiate receptor subtypes.

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